Hematologic Malignancies
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Chronic Lymphocytic Leukemia

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1 Chronic Lymphocytic Leukemia: Who, How, and Where?

Lydia Scarfò and Paolo Ghia

1.1 Introduction

Chronic lymphocytic leukemia (CLL) is characterized by the relentless accumulation in the peripheral blood, bone marrow, and secondary lymphoid organs of clonal B lymphocytes with a distinctive immunophenotype where B-cell markers (CD19, CD23) are expressed along with CD5, with low-level expression of CD20 and surface immunoglobulins (see Chap. 2) [1, 2].

This immunophenotypic profile is so typical that CLL is unique among lymphoproliferative disorders in the sense that tissue biopsy is not needed for a confirmed diagnosis, if all above-mentioned markers are expressed [2].

Despite decades of studies and efforts, CLL pathogenesis is far from being clearly defined. A better understanding of the mechanisms underlying disease onset and evolution has been hampered by the extreme heterogeneity of the disease. This biological heterogeneity is reflected into a remarkably heterogeneous clinical course of patients affected by CLL, including, at the opposite extremes, patients who never require treatment and others who experience a very aggressive disease course, with the vast majority lying in between [3]. The most aggressive clinical phenotype in the spectrum of CLL is represented by the transformation into aggressive lymphomas, usually of the diffuse large B-cell lymphoma (DLBCL) type, defined as Richter syndrome (RS), that occurs in about 2–7% of patients [4].

In this chapter, we will dissect the complex heterogeneity of the disease to define the cellular element leading to CLL (*who*), the mechanisms underlying its onset (*how*), and the environment where these are producing their dreadful effects (*where*).

1.2 Who

1.2.1 Genetic Predisposition to CLL

There is clear evidence for a genetic predisposition in CLL, though its basis remains poorly understood. People of Asian-Pacific descent show lower incidence rates of CLL (average incidence <0.01%), and the overall incidence increases from Eastern to Western countries. Though environmental factors and dietary habits may at least in part influence the risk, lower incidence rates are maintained in the progeny of Asian migrants to the USA.

In 5–10% of cases, CLL occurs in individuals with a family history of CLL and other non-Hodgkin lymphomas (NHLs). The relative risk of

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developing CLL in first-degree relatives of CLL patients in comparison to the general population is increased by 8.5-fold [5–7].

Several genome-wide association studies (GWAS) have identified multiple low-risk CLL susceptibility loci [8–13]. Each locus confers only a mild increase in the risk of developing CLL, but, given their high frequency, they contribute substantially to CLL development, with an overall increase in susceptibility based on the number of alleles identified in each subject. Predisposing single-nucleotide polymorphisms (SNPs) were found in more than 40 genes known to be relevant for transcriptional regulation, B-cell development, differentiation, telomere function, and apoptosis. For instance, an SNP in IRF4 was identified in familial CLL cases leading to reduced IRF4 levels; in vivo models showed that IRF4-deficient mice are prone to develop CLL [14]. An SNP of LEF-1 (a downstream effector of WNT signaling) in familial CLL has been associated with increased LEF-1 levels. Accordingly, LEF-1 expression levels have been reported to be high in CLL and to promote resistance to cell death [15].

1.2.2 Monoclonal B-Cell Lymphocytosis

Precursor states preceding clinically overt disease have been identified for many neoplastic conditions and may help to shed light on key mechanisms leading to disease development.

Monoclonal B-cell lymphocytosis (MBL) is a recently defined condition [16] now included as a distinct entity in the WHO classification of mature B lymphoid neoplasms [17], characterized by the presence of small B-cell clones in the peripheral blood of otherwise healthy individuals. Though a minority of MBL cases show a surface phenotype different from CLL (the so-called atypical CLL and non-CLL), more than 75% carry a CLL-like phenotype and are distinguished from CLL based on a B lymphocyte count $<5 \times 10^9$/l in the absence of other signs or symptoms of lymphoproliferative disorders such as adeno- or organomegaly [18]. CLL-like MBL is further classified according to the size of the clonal population in low-count (B-cell count $<0.5 \times 10^9$/l) and high-count MBL (B lymphocyte count $\geq 0.5 \times 10^9$/l). Low-count MBL is generally discovered through investigational screening studies of healthy individuals, while high-count MBL is detected during laboratory workup for lymphocytosis investigation. This distinction is relevant considering that high-count MBL has been identified as preneoplastic stage of CLL, resembling the link between monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma, with a definite risk of progression into a frank leukemia around 1% per year [19, 20].

Low-count MBL is more frequent in elderly people and its biologic features, such as the immunoglobulin gene repertoire usage, are generally different from CLL, even if compared with early stage disease, though it may share with CLL similar gross chromosomal aberrations [21–23]. In a relevant proportion of low-count MBL, multiple B-cell clones (i.e., oligoclonality) [24] have been reported along with oligoclonal T-cell expansions [22]. This wide immune dysregulation, along with the increased prevalence of low-count MBL with age, suggests that immune senescence rather than tumorigenesis may explain this condition.

At variance, biologic characteristics of high-count MBL are very similar to early stage CLL, showing the same biased usage of immunoglobulin genes and carrying the same somatic mutations on CLL driver genes (see next) [23]. From a clinical standpoint, high-count MBL shares also with CLL an increased risk for infection [25] and for second primary malignancies [26], which further supports a strict relationship.

Traditional CLL prognostic factors have been investigated in CLL-like MBL to define the risk of progression into overt CLL requiring treatment with unsatisfactory results. The only factor consistently associated with risk of progression from MBL to CLL is the size of the CLL clone in the peripheral blood [19, 21]. Accordingly, the progression rate of high-count MBL into CLL requiring treatment is around 1–2%, while the risk of progression of low-count MBL is negligible, if any.

Large prospective investigations with prolonged follow-up and comprehensive biologic assessments are still needed to clarify this issue.

1.2.3 Cell of Origin

In line with the biological and clinical heterogeneity of the disease, the cell of origin for CLL is still matter of debate as no unifying pathogenetic mechanisms have been so far identified. A complex interplay between genetic alterations and microenvironmental stimuli is thought to lead to full-blown disease but the relative weight of the two components remain to establish and in particular the sequencing of the events in the leukemogenic process. In the last decades, several hypotheses have been generated that can be summarized into two opposite, though potentially interrelated, scenarios.

On the one side, recent results derived from mouse models suggest that very early genetic and epigenetic alterations in CLL may occur at the hematopoietic stem cell (HSC) level. Using xenotransplant models, CLL HSCs were able to recapitulate the disease onset and evolution starting from an expansion of polyclonal B-cell progenitors to the appearance of oligoclonality and the occurrence of MBL though without the development of a full-blown CLL [27]. Along this line of evidence, CLL-driver mutations were found in the hematopoietic compartment of the bone marrow of CLL patients and confirmed to be present in the HSCs with the potential of being carried on into the B-cell lineage where they may contribute to the leukemia development [28].

On the other side, the putative normal counterpart of CLL clones has been identified in an antigen-experienced precursor, resembling memory B cells. Phenotypic data are in line with this view documenting CD27 expression (a memory B-cell marker), high expression of CD23, CD25, CD69, and CD71 that are usually upregulated after antigen encounter, while lower expression of FCγRIIB, CD79b, and IgM/IgD is concordant with downregulation of these markers upon cellular activation [29]. The discovery that, in >50% of cases, CLL cells carry mutations in their IGHV genes [30] was also brought up to support the role of antigen exposure in CLL development and to argue that CLL cells might be derived from post-germinal center (GC) B cells at least in a proportion of cases. More recently, transcriptome analyses found a stringent similarity between CLL and normal mature $CD5^+$ B cells, the originally proposed cell of origin for CLL [31, 32]. CD5 by itself can be a marker of B-cell activation, at least in humans, rather than identifying a distinct cell lineage. Based on the presence or the absence of IGHV gene somatic mutations, the cell origin for CLL might then be different. Mutated IGHV CLL clones (i.e., those where immunoglobulin sequences show <98% identity to germline) resemble a post-GC, T-cell-dependent memory $CD5^+CD27^+$ B-cell population. Conversely, unmutated CLL clones (≥98% identity) appear to derive from a small fraction of $CD27^+$ unmutated memory B cells that attained a memory phenotype after being activated in a T-, GC-independent fashion.

1.3 How

1.3.1 Mechanisms of Leukemogenesis

A number of *intrinsic* gene defects, either gross chromosomal aberrations or point somatic mutations, have been reported in CLL though again none of them characteristic of the disease [3]. In addition, a number of pathways have been described that appear to be "constitutively" active in the disease in the absence of any known genetic abnormalities thus suggesting the existence of *extrinsic* stimuli acting on the leukemic clone leading to its activation. Among others, the best studied so far is the one acting through the clonal B-cell receptor (BcR) that led to a therapeutic exploitation with the approval of drugs targeting the molecules on the downstream pathway [33–40]. Additional pathways, such as those originating from the Toll-like receptors (TLR), have also been shown to cooperate in shaping the functional activation of the leukemic clones [41].

The interplay between intrinsic and extrinsic events remains to be established fueling a classic chicken–egg debate. Are the genetic abnormalities coming first and predisposing a particular B cell equipped with a specific BcR to react abnormally to its cognate antigen and paving the way to leukemic transformation? Or, conversely, is the particular stimulation occurring between a certain BcR and its antigen leading to protracted activation of pathways and genes that may result in the occurrence/selection of particular gene defects?

1.3.2 Genetic Defects

Conventional karyotype banding and fluorescent in situ hybridization (FISH) analysis have been applied since a long time in CLL and laid the ground for the current basis of prognostication and response prediction for the management of patients [42].

At variance with other hematological malignancies, recurrent chromosomal translocations are extremely rare in CLL and mainly limited to t(14;18), involving BCL2 and immunoglobulin heavy chain (2% of cases).

In contrast, up to 80% of CLL patients at diagnosis show FISH-detected aberrations, the most common ones being del(13q), trisomy 12, del(11q), and del(17p). These aberrations have been arranged in a prognostic hierarchical model by Dohner et al. [42], where patients carrying del(13q) have the most favorable outcome in terms of progression-free and overall survival, while del(17p) confer the poorest survival, followed by del(11q), trisomy 12, and normal karyotype (i.e., no aberrations detected by standard FISH panel) (see also Chap. 4).

Del(13q) is found in about 55–60% of patients and it is associated with favorable clinical course when detected as sole abnormality. The deleted region causes the loss of two regulatory microRNAs, i.e., miR15a and miR16-1 [43]. miR15a and miR16-1 inhibit the transition from G0 to G1 phase in cell cycle and negatively control BCL2 activity in normal and leukemic cells [44]. Mouse models and in vitro studies showed that this early lesion causes cell cycle and BCL2 hyperactivation and favors leukemic cell survival [45]. Interestingly, only around 40% of the genetically modified animals, missing the miRNAs or larger portion of the chromosome, developed clonal populations (MBL, CLL, or DLBCL) suggesting that additional elements are required for the appearance of a full-blown leukemia [45]. Del(13q) has been described also at similar frequencies in low-count MBL, again reinforcing the concept that the lesion is associated with the acquisition of the CLL phenotype rather than the progression into a clinically relevant disease [22].

Trisomy 12 (found in 10–16% of CLL patients) has been associated with increased incidence of secondary tumors and Richter's transformation [46]. It is frequently detected in association with NOTCH1 mutation (see below), but the precise molecular mechanism behind this frequent abnormality remains unknown (see Chap. 10) [47].

Del(11q) is found in about 6–27% and the deleted region includes the ataxia telangiectasia mutated (ATM) tumor suppressor gene, playing a key role in response to DNA damage [48]. In some cases, the deletion may encompass also baculoviral IAP repeat containing 3 (BIRC3) gene, a negative regulator of the noncanonical NFKB pathway. Cases associated with these aberrations show genomic instability and follow an unfavorable clinical course with early progression [49].

Del(17p) is rare at diagnosis (up to 3.5–5%), but its frequency progressively increases at relapse and in chemorefractory or transformed disease (see Chap. 4). The second TP53 allele is found somatically mutated and thus functionally inactivated in 80% of cases with del(17p) [50]. The inactivation of the TP53 pathway causes genomic instability and is associated with higher genomic complexity, being implicated in poor responses to DNA-damaging chemotherapeutic agents [51–53].

More recently, the use of next-generation sequencing (NGS) has led to uncover somatic mutations in many novel putative disease-driving pathways and has allowed to appreciate the genomic complexity behind the homogenous phenotypic profile.

Again, also in the case of NGS, no universal CLL-related lesions or altered pathways have been identified, while a number of putative CLL drivers have been found recurrent in at best 10–15% of cases [54–58]. They appear to associate with several molecular mechanisms, including DNA-damage response, RNA processing, NOTCH pathway, BcR signaling, and the B-cell transcriptional program and chromatin maintenance. The inflammatory response, mitogen-activated protein kinase (MAPK)–extracellular signal-regulated kinase, and MYC-related signaling are other relevant pathways affected by mutations. The most frequent and intriguing mutated genes are briefly described below.

SF3B1 Splicing factor 3b subunit 1 (SF3B1) encodes a crucial component of the spliceosome machinery and most mutations probably affect the interaction between SF3B1 and RNA [55, 59, 60]. It is worth noting that up to 30% of CLL patients may show mutations in genes involved in RNA splicing suggesting that RNA splicing deregulation may represent a common mechanism of disease pathogenesis in CLL [61].

NOTCH1 Mutations have been reported in about 10% of CLL cases at diagnosis and found to be associated with unmutated IGHV genes and trisomy 12 [62, 63]. Different mutations lead to the deregulation of the intracellular portion of NOTCH1 receptor, causing the activation of NOTCH1 transcriptional program [64–66].

BIRC3 It is involved in proteasomal degradation of MAPK3K14 that leads to noncanonical NF-κB pathway activation. Mutations in this gene impair its E3 ubiquitin ligase activity, conditioning constitutive NF-κB activation [49]. Other genes related to NF-κB pathway that have been shown to be recurrently mutated in CLL include MYD88 and NFKBIE [67].

MYD88 Mutations in this gene are detected in 2–5% of CLL patients [54, 68]. MYD88 is an adaptor protein involved in the regulation of toll-like receptor (TLR) pathways, and its mutation (detected also in other B-cell lymphoproliferative disorders like lymphoplasmacytic lymphoma and DLBCL) leads to multiple target activation, including STAT3 and NF-κB p65 subunit [69, 70].

Whole exome (WES) and whole genome (WGS) sequencing studies shed also light on the clonal architecture during the disease course. Investigation on sequential samples documented that early events [del(13q), del(11q), trisomy 12, and MYD88 mutations] are preferentially clonal and are considered CLL initiators, while late events (ATM, SF3B1, and TP53 aberrations) are detected at subclonal level [57, 58]. In this regard, CLL-related lesions seem to be acquired in a temporally defined order, instead of being random events [71]. Repetitive patterns of co-occurrence and mutually exclusive lesions have been identified, suggestive of nonredundant mechanisms shaping clonal evolution in each case [72].

The clonal dynamics is even more relevant if correlated with the development of treatment resistance, because specific treatments apparently elicit different clonal evolution patterns based on fitness advantage of subclonal populations. The clearest model is represented by the selection of TP53 aberrant clones in patients exposed to chemoimmunotherapy combinations [58, 73], while patients developing resistance to ibrutinib (the first-in-class BTK inhibitor) experience the expansion of BTK- or PLCγ2-mutated clones developing over treatment [74–77].

The role of each recurrent mutation in CLL pathogenesis, their prognostic significance, and in particular their predictive value for response to standard and novel agents need to be validated in *in vitro* and *in vivo* functional studies and analyzed in larger prospective CLL patient cohorts, in order to translate deeper understanding of disease-related mechanisms in clinical benefit for CLL patient management.

1.3.3 B-Cell Receptor

It is now widely accepted by the scientific community that in all cases CLL cells have experienced antigenic stimulation through the

BcR. This notion is supported by a number of experimental evidences, highlighting the relevant role of the immunoglobulin receptor in CLL pathogenesis and, more recently, by the great efficacy shown by novel agents targeting molecules on the BcR pathway that have been recently approved for the treatment of CLL.

A functional BcR is required for the survival of normal mature B cells [78] and it is usually preserved in mature B-cell malignancies. BcR structure is composed of a ligand-binding immunoglobulin (IG) molecule coupled with CD79A/CD79B heterodimer. After antigen binding, BcR signaling is usually initiated by Lyn-dependent phosphorylation of CD79A and CD79B that leads to binding and activation of SYK. A signalosome, consisting of BTK, AKT, PI3K, PLCγ2, and BLNK among the others, is recruited by SYK and promotes downstream signaling cascade, including diacylglycerol (DAG) and phosphatidylinositol (3,4,5)-trisphosphate (PIP3) generation, ERK, and NF-κB activation (Fig. 1.1). In normal B cells, affinity maturation in secondary lymphoid organs upon antigen encounter is a key process and includes somatic hypermutation (SHM) of immunoglobulin heavy variable (IGHV) genes. It consists essentially of single base substitutions that improve the affinity for the antigen.

It has long been known that the immunoglobulin (IG) gene repertoire expressed on leukemic cells by CLL patients is highly skewed suggesting selection through antigenic binding during the natural history of the disease [30, 79, 80]. Later on, we learnt that CLL patients can be distinguished in two subgroups based on the presence (<98% germline identity) or the absence (≥98% germline identity) of SHMs in the IGHV genes [30]. The SHM status of the clonotypic IGHV genes is a strong and independent prognostic factor for CLL clinical course: cases with unmutated IGHV genes (about 40% of CLL at diagnosis) follow a dismal clinical course characterized by early progression and reduced overall survival if compared to patients with somatically mutated IGHV genes (about 60%) [81, 82]. The

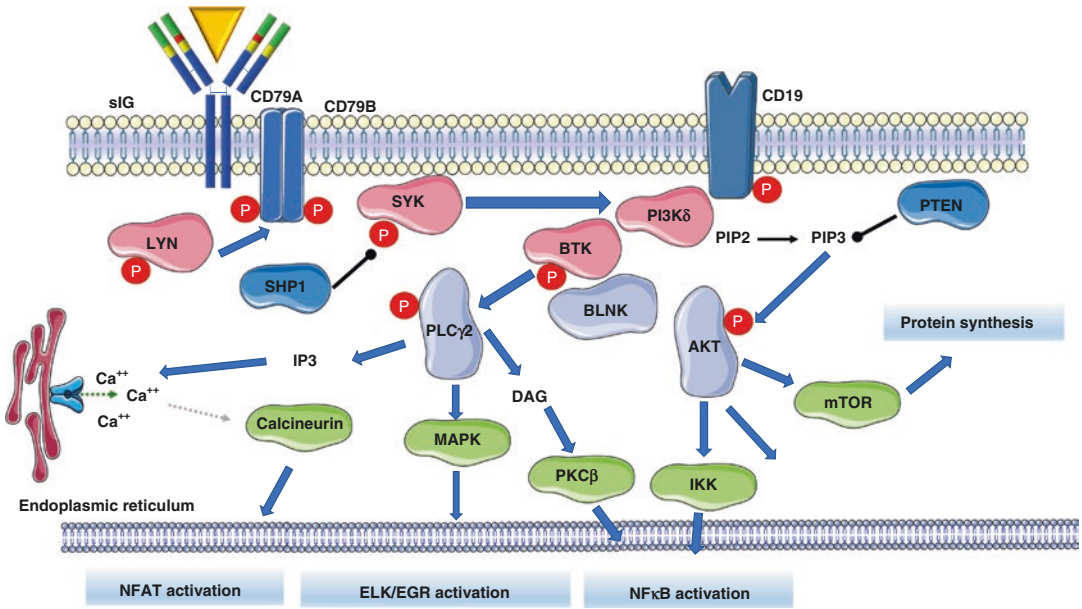

Fig. 1.1 B-cell receptor signaling. BCR triggering by antigen binding induces the activation of upstream kinases (i.e., LYN and SYK) which phosphorylate CD79A and CD79B. This event leads to the activation of other upstream kinases, i.e., BTK and PI3Kδ, followed by the activation of downstream pathways, including PLCγ2, calcium signaling, MAPK/NFAT, and NFκB pathway. Kinases for which targeted inhibitors have been tested in clinical trials are depicted in red. The figure was produced using Servier Medical Art: www.servier.com

evidences supporting the key role of IGHV genes in CLL prompted further immunogenetic analysis trying to dissect the mechanisms behind it [83]. The variable domain of IG genes contains three highly variable regions interacting directly with the antigens and thus called complementarity-determining regions (CDRs). Among these three, the one at the junction of the IGHV, IGHD, and IGHJ genes (called HCDR3) has the highest variability, and the probability to find identical BcR IG in different B-cell clones by chance alone is extremely remote ($\sim 10^{-12}$). Cooperative international efforts collecting thousands of CLL IG gene sequences demonstrated that up to 30% of patients carry subsets of quasi-identical (or "stereotyped") BcR sharing similar HCDR3 [84–86]. Stereotyped BcR are currently defined by IGHV–IGHD–IGHJ gene rearrangement sequences carrying IGHV genes of the same clan, sharing identical HCDR3 lengths and amino acid positions within the HCDR3 region; finally, they must share at least 50% amino acid identity and show 70% similar amino acid physicochemical properties [87]. Hundreds of stereotyped subsets, each defined by a unique HCDR3 motif, have been identified, with 19 major subsets that contained 20 or more CLL cases [88, 89].

These subsets of stereotyped BcR represent biologically and clinically distinctive entity among CLL patients. CLL cases belonging to the same stereotyped subset share not only immunogenetic features but also genetic aberrations, and epigenetic and transcriptomic profiles and display similar responses to BcR triggering that is highly distinctive for any given subset [90–97].

Recent multi-institutional international series reported that stereotyped subsets share also clinical features not only in terms of baseline characteristics (including age, gender distribution, and disease burden at diagnosis) but also in terms of risk for and time to progression and eventually outcome. In this regard, the characterization of BcR stereotypy is able to refine prognostication beyond the traditional SHM-based classification [98].

For instance, subset #2 cases, the largest stereotyped subset overall and one with the worst prognosis, account for 2.5–3% of all CLL and are highly enriched with SF3B1 mutations, potentially explaining its high disease burden at diagnosis and its aggressive clinical course [97].

On the other side, subset #4 cases, the largest subset among mutated CLL cases, follow an indolent clinical course and associate with favorable genetic aberrations, mainly del(13q), being devoid of the poor-prognosis genetic aberrations.

All in all, the demonstration of BcR stereotypy in CLL further strengthens the key role for antigen selection in the natural history of the disease but also in shaping the clinical behavior of the individual patients.

In terms of antigenic elements acting in the disease, foreign or autoantigens have been identified that are able to interact and stimulate leukemic BcRs, with heterogeneous functional consequences ranging from anergy to full activation [99–102]. Response after BcR engagement may vary in different CLL cells and correlate with prognosis. Anergic features after BcR triggering (mainly represented by reduced calcium influx, reduced ERK phosphorylation, and downstream kinases activation) have been demonstrated in about 50% of CLL cases and found to correlate with indolent clinical course [103]. On the opposite side, CLL cells characterized by intense BcR activation upon antigen binding (increased calcium influx and increased MAPK pathway phosphorylation) are typically associated with unfavorable outcome [104].

In vitro and *in vivo* findings have recently challenged this traditional perspective of antigenic stimulation as the existence of a so-called *cell-autonomous signaling* restricted to CLL cells has been reported [105]. This appears to be the result of the interaction of the leukemic BcR IG with epitope(s) of the same or adjacent BcR IGs that seem to be distinct for different subsets of patients and capable of inducing intracellular activation in the absence of a classic antigen binding [106]. It remains to be clarified how this auto-recognition mechanism may cooperate with the classic antigenic stimulation in the onset and maintenance of the disease.

1.4 Where

1.4.1 Microenvironmental Stimuli

The relevance of the BcR stimulation in CLL is paradigmatic of the dependency of leukemic cells on survival and proliferative signals they receive from the surrounding microenvironment. These interactions occur primarily in secondary lymphoid organs as witnesses by the evidence that the BcR-related and the NFKB pathways are "constitutively" activated in the lymph nodes, in contrast to peripheral blood and bone marrow [107]. Leukemic cells promote the development of a specialized niche that derives from the active interactions with a number of soluble factors and accessory cells. This equilibrium is dynamic and it is actively shaped by CLL-derived signals. How relevant this interaction is for CLL cells is underscored by the fact that primary CLL cells show only limited survival in vitro undergoing apoptosis unless cytokines and supportive cells are provided to the culture system [108].

Historically, CLL was considered a disease of resting B cells, where leukemic cells have a limited proliferative potential and tend to accumulate because of an increased resistance to death. Even though most CLL cells in the peripheral blood are in a resting state, small populations of proliferating cells could be identified in tissue reservoirs that fuel the disease bulk, the so-called *proliferation centers* where likely the antigen encounter occurs [109]. Within proliferation centers, large, proliferating CLL cells come in contact with accessory cells that are recruited through the release of cytokines and chemokines (Table 1.1) [125]. These cells such as T cells, monocytes, nurse-like cells (NLCs),

Table 1.1 Key CLL microenvironmental interactions

Receptor	Ligand	Cellular interaction	Function	References
CXCR4	CXCL12	CLL cells–NLCs	• Induces chemotaxis of CLL cells • Promotes survival of CLL cells	[110, 111]
CXCR5	CXCL13			
CCR1 and CCR5	CCL3	T cells, monocyte/macrophages–CLL cells	• Recruits T cells and monocyte/macrophages to tissue sites for interactions with CLL cells	[112, 113]
CCR5	CCL4			
BAFFR	BAFF	CLL cells–NLCs	• Promote survival of CLL cells	[114]
BCMA/TACI	APRIL			
CXCR3	CXCL4,1,9,10,11	CLL cells–NLCs	• Regulates cell proliferation, survival, and migration	[115]
CD31	CD38	NLCs–CLL cells	• Supports interactions and differentiation	[116]
RAGE	HMGB1	NLCs–CLL cells	• Stimulates NLC differentiation	[117]
CD40	CD40L	CLL cells–T cells	• Promotes survival of CLL cells	[118, 119]
PD-1	PD-L1	T cells–CLL cells	• Inhibits T-cell responses	[120, 121]
CCR4	CCL22	CLL cells	• Recruits T cells	[118, 119]
VCAM-1	VLA-4	BMSCs–CLL cells	• Organizes CLL-cell trafficking and tissue homing	[122]
ETAR	ET-1	CLL cells–endothelial cells	• Promotes CLL survival and drug resistance	[123]
LTβR	LTαβ	FDCs–CLL cells	• Guides CLL positioning within lymphoid follicles and leukemia progression	[124]

NLC nurse-like cell, *BAFF* B-cell activating factor, *APRIL* a proliferation-inducing ligand, *RAGE* receptor for advanced glycation end product, *HMGB1* high-mobility group box 1, *PD-1* programmed cell death protein 1, *PD-L1* PD-1 ligand, *VCAM-1* vascular cell-adhesion molecule-1, *VLA-4* very late antigen-4, *BMSC* bone marrow stromal cell, *ETAR* endothelin subtype A receptor, *ET-1* endothelin 1, *LTβR* lymphotoxin beta receptor, *LTαβ* lymphotoxin alpha beta, *FDC* follicular dendritic cell

stromal cells, and mesenchymal-derived stromal cells (MSCs) in turn deliver antiapoptotic signals and proliferative stimuli to CLL cells in a vicious loop [110, 114, 126].

Gene expression profiles of CLL cells from different tissue compartments confirmed that the lymph node is the key site of CLL-cell activation and tumor proliferation. CLL cells derived from nodal compartment show a relevant increase in BcR and NF-κB activation in comparison to other sites, suggesting an ongoing antigenic stimulation and a constant microenvironmental interaction [107, 127].

Studies using a deuterium oxide (^2H_2O) in vivo labeling method have been used to quantify and characterize CLL cells capable of active proliferation [128–130]. Primary samples from lymph node, peripheral blood, and bone marrow confirmed that the largest fraction of newly born cells is harbored in the lymph node and enriched of $CXCR4^{dim}$ $CD5^{bright}$ cells. These cells are highly proliferating and can egress to the circulation losing their proliferative potential and becoming progressively $CXCR4^{bright}$ $CD5^{dim}$ [129]. Such studies brought to evidence that CLL cells have often substantial birth rates, varying from 0.1% to greater than 1.0% of the entire clone per day [128]. This suggests that the disease is a dynamic process composed of cells that proliferate and die, and not only of cells resistant to undergo apoptosis as classically believed, with higher proliferation rate being associated with progressive disease course [130].

1.4.2 Cellular Components

The proliferation centers are the site where most of the action is happening in CLL and where a number of accessory cells cooperate with the leukemic clone to sustain its proliferation and survival. Among others the following have been studied more in detail.

T cells The total number of both $CD4^+$ and $CD8^+$ subsets is increased in the peripheral blood but, more relevantly, the TCR repertoire is skewed in both compartments, with oligoclonal populations being detected to suggest particular selection occurring also in T lymphocytes [131–133]. We now know that T cells are profoundly influenced by CLL cells in the nodal microenvironment. On the one side, activated $CD4^+$ T cells are recruited in the proliferation centers through CCL22 secretion by leukemic cells [118, 119]. $CD4^+$ T cells are then subverted to promote CLL expansion providing CD40L costimulation and pro-survival Th2 cytokines that in turn activate ERK, STAT3, and NF-κB signaling and increase CLL-cell proliferation [134].

On the other side, $CD8^+$ T cells exhibit abnormal gene expression profile, characterized by downregulation of cytoskeletal genes causing impaired immunologic synapse formation and vesicle trafficking, leading to an impaired effector function, along with abnormal expression of exhaustion-like surface markers such as PD-1 [120, 121, 135]. This is in keeping with the long-standing notion that T-cell function is disrupted and dysregulated in CLL probably due to the interaction with the leukemic B lymphocytes, likely leading to the known predisposition to infections of the patients.

Nurse-Like Cells NLCs differentiate from monocytes if cocultured with CLL cells [111] and show an M2-like phenotype of tumor-associated macrophages (TAMs) by gene expression analysis, known to exert a pro-tumoral effect in solid tumors [115, 117]. According to this, they affect CLL activation and cell survival through different mechanisms essentially promoting the expression of anti-apoptotic genes belonging to the BCL2 family. They tightly regulate CLL-cell homing through chemokine receptors and adhesion molecules. NLCs, together with mesenchymal stromal cells, secrete C-X-C motif ligand 12 (CXCL12) and CXCL13 attracting CLL cells through CXCR4 binding [136]. CLL-cell chemotaxis and survival are promoted also by expression of B-cell activating factor (BAFF) and proliferation-inducing ligand (APRIL), TNFα family members that are expressed on NLCs and engage their receptors (BCMA, TACI, and BAFF-R) on leukemic cells [114].

In addition, NLCs promote BcR and NF-κB signaling activation in CLL cells. CLL cells secrete high levels of CCL3 and CCL4 following BcR stimulation, and higher CCL3 and CCL4 plasma levels have been correlated with inferior clinical outcome [112, 113]. They are also currently investigated as predictive markers of response in patients treated with novel BcR inhibitors, as these cytokines tend to decrease in responders and to increase again at time of relapse.

NLCs express activating molecules including CD31, that is the ligand for CD38, a well-recognized prognostic factor in CLL that correlates with shorter progression-free survival [116]. CD38 ligation prolongs CLL-cell survival and favors leukemic cell proliferation with its expression being regulated by the microenvironment and serving as a marker of (recent) activation of the leukemic clone.

Mesenchymal Stromal Cells MSCs are key components of the normal bone marrow architecture. In CLL, they protect leukemic cells from spontaneous and drug-induced apoptosis and regulate CLL-cell trafficking and homing [108]. In a bidirectional crosstalk, MSCs are activated by CLL cells inducing protein kinase C beta II (PKCβII) expression, AKT, and NF-κB pathway activation [122].

1.5 Conclusions

These are exciting times for CLL scientists, physicians, and patients as, in the last decade, inconceivable and unprecedented progresses have been made in understanding key mechanisms in CLL onset and progression.

A preneoplastic condition for the disease (i.e., MBL) has been identified that may help us to shed light on the initial phases of CLL occurrence. This will allow to identify very early molecular events responsible for CLL development but also to understand which factors define the thin red line between clinically overt disease and preclinical condition.

The introduction of high-throughput sequencing techniques has increased our knowledge of the genomic landscape of CLL cells and introduced the concept of clonal architecture and evolution, proving that both are influenced by any therapeutic intervention and this should be taken into account when selecting time and type of treatment in CLL patients.

We gained further knowledge on the role of BcR signaling and accumulated several evidences supporting its key role in CLL development and progression. This led, for the first time in CLL history, to a targeted treatment approach, with novel inhibitors now available in the clinical practice for our CLL patients.

That notwithstanding, there are many open questions in CLL pathogenesis that need to be addressed and are currently filling the research agenda of the next years:

- Are we getting closer to identify the cell(s) of origin?
- Are we able to reconcile the complex genetic landscape discovered by NGS studies to identify common pathways and potential therapeutic targets?
- With novel agents available are our "old" consolidated prognostic markers still valid? Do we need novel predictive and/or prognostic biomarkers?

Stay tuned for more!

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Part II

Diagnostics

2 Laboratory Diagnosis of Chronic Lymphocytic Leukaemia

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2.1 Introduction

The World Health Organization (WHO) defines chronic lymphocytic leukaemia (CLL) and small lymphocytic lymphoma (SLL) as a neoplasm composed of monomorphic B-lymphocytes that usually co-express CD5 and CD23. The term SLL is used for non-leukaemic cases with the tissue morphology and immunophenotype of CLL. In the absence of extramedullary tissue involvement, there must be >5 × 10^9/L monoclonal B-lymphocytes in the peripheral blood for a diagnosis of CLL; otherwise, the disorder is classified as monoclonal B-cell lymphocytosis (MBL) [1–4].

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The incidence of CLL varies according to age and location, with lower frequency in WHO Asia and Africa regions (age-adjusted incidence <4/100-thousand/year [http://gco.iarc.fr/today/home]) compared to Europe and North America (age-adjusted incidence >5/100-thousand/year). In these regions, CLL accounts for approximately 1% of all new cancer cases with a median age at diagnosis of 70 years, male:female ratio of nearly 2:1 and 5-year relative survival of approximately 85% [5, 6].

Whole exome sequencing has identified 55 driver events with more than 90% of CLL cases demonstrated to have at least one driver but there is no pathognomonic molecular abnormality [7]. The diagnosis of CLL continues to rely on the morphological and immunophenotypic features which are usually highly characteristic but vary in a proportion of cases and can show some overlap with leukaemic manifestations of some other B-lymphoproliferative disorders, particularly mantle cell, marginal-zone and lymphoplasmacytic lymphomas [1–4].

2.2 Full/Complete Blood Count

The presentation, course and outcome in CLL are highly variable but the majority of newly diagnosed cases in North America and European regions present with early stage asymptomatic disease after an incidental finding of

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lymphocytosis [8]. The approach to investigating lymphocytosis will vary between different healthcare facilities and, if there is no cytopenia, lymphadenopathy or B-symptoms, it will often be appropriate to exclude infectious causes or a transient lymphocytosis before investigating for a lymphoid neoplasm. The International Workshop on Chronic Lymphocytic Leukaemia (IWCLL) guidelines for diagnosis of CLL require that the monoclonal B-cell lymphocytosis persists for at least 3 months [1]. In some centres, the initial investigation of the lymphocytosis will be through a flow cytometry screening tube/panel to enumerate T/NK-cell subsets and assess B-cell clonality [9], but in most cases a morphological assessment will precede immunophenotyping. Immune haemolysis or thrombocytopenia can be a presenting feature in patients with otherwise early stage disease.

2.3 Morphology

The leukaemia cells found in the blood smear are characteristically small, mature lymphocytes with a narrow border of cytoplasm and a dense nucleus lacking discernible nucleoli and having partially aggregated chromatin. Ruptured lymphocytes (known as smudge, smear or basket cells) are evident on the blood film in the vast majority of CLL cases, and reported to be associated with reduced levels and abnormal arrangement of vimentin, an intermediate filament protein critical for cellular integrity [10, 11]. Larger, atypical lymphocytes or prolymphocytes (medium-sized lymphoid cells with basophilic cytoplasms and prominent nucleoli) may be seen but should not exceed 55% [1].

2.4 Immunophenotype

The IWCLL guidelines require that CLL cells co-express the surface antigen CD5 together with the B-cell antigens CD19, CD20 and CD23 [1], while the WHO criteria indicate that CLL cells usually co-express CD5 and CD23 but that some cases may have an atypical phenotype with lack of CD5 or CD23 [3]. Although CD5 is often the primary marker used to initiate differential diagnosis of CLL from other B-cell disorders, this marker is expressed in mantle cell lymphoma and in a varying proportion of other lymphomas, while the level of expression may be weak in CLL and therefore other markers may be more informative.

CD20, surface immunoglobulin and CD79b expression levels are typically weaker in CLL than in all other B-lymphoproliferative disorders and normal B-cells. CD20 and CD79b show better discrimination of CLL cells from normal B-cells than CD5 and other B-cell markers [12]. Reduced CD20 expression has historically been assessed using the FMC7 antibody, which is a weakly expressed epitope of CD20, such that cases with detectable FMC7 expression also have strong expression of CD20, while FMC7 is not detectable in cases with weak or no CD20 expression [13]. Diagnostic algorithms that consider strength of CD20 have demonstrated redundancy in assessing both markers [14] but many centres still include both CD20 and FMC7 because the dichotomous results provided by FMC7 may be easier to interpret in diagnostic algorithms. CLL cells also have reduced expression of immunoglobulin heavy chains IgM and IgD compared to normal B-cells, although IgD may be more likely retained than IgM [15]. B-cell receptor signalling is central to CLL pathogenesis and there are functional differences after activation of IgM compared to IgD, and therefore analysis of both heavy chain isotypes may be informative [16]. Class-switched CLL occurs infrequently and is often associated with specific IGHV/IGKV gene combinations [17]. Analysis of surface immunoglobulin may be affected by levels of serum immunoglobulin, number and volume of prestain washing steps as well as the antibodies used, and therefore it can be difficult to interpret and has weaker specificity for discriminating CLL than CD79b [18]. The expression levels of CD20, CD79b and sIg show a continuous distribution in CLL and therefore each centre must determine the range of expression on normal polyclonal mature B-cells in order to define "weak" expression typical of CLL.

CD23 is a low-affinity receptor for IgE that can be cleaved from cell surfaces to yield soluble CD23 (sCD23) that has cytokine-like activities [19]. Prior to the characterisation of mantle cell lymphoma as a diagnostic entity, the lack of CD23 expression was noted as a characteristic of a small series of lymphoma patients with *t*(11;14) [20]. CD23 has been used in the differential diagnosis between MCL and CLL/SLL by immunohistochemistry [21] and in the flow cytometry scoring systems [22] for more than two decades. CD23 expression is closely associated with progression of CLL cells through cell cycle made evident by increased CD23 expression in proliferation centres [23], close correlation between the strength of CD23 and Ki67 expression by flow cytometry [24] and loss of CD23 expression coinciding with maximal inhibition of proliferation during ibrutinib treatment [25]. CD23 expression may be lost during sample transit, with potential false-negative results in samples that are more than 24 h old. As CD23 expression is relevant to the biology of CLL, diagnosis of CD5+CD23- B-LPD CLL should be made with caution, particularly if the sample is <24 h old on analysis. Furthermore, CD23 is not restricted to CLL and weak expression may be detected in 5–50% of MCL depending on the diagnostic approach [21, 26–30].

CD200 is a type I membrane glycoprotein expressed by various cell types, including mature B-cells, a subset of T-cells, thymocytes, endothelial cells and neurons. CD200 is reported to play an important role in immunosuppression and regulation of antitumour activity. CD200 is typically expressed in CLL but not in mantle cell lymphoma, and has therefore been identified as a marker that can facilitate the differential diagnosis of CLL/SLL vs. MCL [14, 26, 31–33]. However, a small subset of MCL cases express both CD200 and CD23 [34, 35]. In our centre, of 115 cases of MCL confirmed by FISH for the *t*(11;14), CD23 expression was detected in 23/115 (20%) and CD200 expression in 19/115 (17%) with CD23 and CD200 co-expression in 9/115 (8%) and therefore CD23/CD200 expression cannot definitively differentiate CLL/SLL from MCL.

CD43 is a sialoglycoprotein, originally called leukosialin [36] that is present at high levels in the majority of leukocytes but absent in mature B-cells. CLL/SLL and Burkitt lymphoma typically have strong expression, with weaker expression typical in mantle cell lymphoma. CD43 expression is rare in follicular lymphoma and variable in other B-lymphoproliferative disorders [37]. CD43 expression has been reproducibly identified as one of the best markers to differentiate between CLL cells and normal mature B-cells and is therefore routinely incorporated in assays for minimal residual disease (MRD) [38–43]. In our experience, there is a significant difference in the level of CD43 expression between CLL and MCL but it is not straightforward to use this information in a simple diagnostic algorithm. However, CD43 is one of the top three markers (with CD20 and ROR1) for differential diagnosis between CLL and post-germinal centre (GC) B-cell disorders (LPL/WM and marginal-zone lymphomas) and contributes substantially to refining diagnosis in CD5+ B-lymphoproliferative disorders [14, 35, 44, 45].

ROR1 was initially identified as a CLL-specific marker in gene-expression profiling studies [46, 47], and uniform expression by CLL cells but not normal mature B-cells has been confirmed at the protein level [48–50]. ROR1 is also expressed at specific stages of B-cell differentiation (CD34-TdT- progenitors) and in some cases of B-lineage acute lymphoblastic leukaemia [51]. Mantle cell lymphoma shows weak expression in a proportion of cases but there is little or no detectable expression in other B-cell disorders [52]. ROR1 does not contribute substantially to the differential diagnosis between CLL and MCL but has high specificity for distinguishing CLL from post-GC B-LPD and is therefore recommended in the diagnostic assessment by the European Research Initiative on CLL (ERIC) and European Society for Clinical Cell Analysis (ESCCA) groups [53]. Expression persists during treatment [50], and ROR1 has been included in commercial kits for minimal residual disease detection.

Markers assessed primarily by immunohistochemistry include Cyclin D1, IRF4 and BCL6.

In contrast to Cyclin D1 translocations which are by definition absent in CLL/SLL, Cyclin D1 expression can be detected in 10–20% of cases without evidence of CCND1–IGH translocation or SOX11 expression. The Cyclin D1 expression seen in CLL/SLL is weak and restricted to proliferation centres [54–56]. IRF4 is a transcription factor that regulates the transition of B-cells to plasma cells [57] and is one of the best markers for identifying proliferation centres in CLL [58], see Fig. 2.1. BCL6 is a transcriptional repressor required for the formation and maintenance of germinal centres and used in the classification of B-LPD as GC vs. post-GC [59] and may be helpful in some cases to discriminate CLL from a GCB disorder.

Markers that should not be detected in CLL and may be helpful as negative controls in diagnosis include CD10, CD103 and CD138. CD10 is routinely included in diagnostic panels for positive identification of germinal centre B-cell disorders and acute leukaemia. It is recommended as a negative control for CLL diagnosis by the ERIC/ESCCA group because there is no reported CD10 expression in typical CLL but it is expressed in occasional cases of MCL [60]. CD103 is one of the essential markers for diagnosis of hairy cell leukaemia (HCL) and should also be absent in CLL [3, 4]. Approximately, 5% of B-LPD will have a second monoclonal B-cell population present [61, 62], and in some cases there will be co-existent CLL and HCL [63]. The co-existence of CLL and HCL is infrequent but may be helpful to exclude in cases with modest CD5+ monoclonal B-cell lymphocytosis and cytopenia. Partial plasma cell differentiation evident through strong CD38 expression and weak CD138 expression would be more consistent with WM/LPL, of which a variable proportion of cases co-express CD5 and/or CD23 [64–66].

2.5 CLL Diagnostic Algorithms Based on Immunophenotype

There is no single marker that can discriminate CLL from other B-LPD, and diagnosis involves the assessment of the expression profile of several different markers in combination. Data reduction approaches to simplify the assessment of multiple markers into a dichotomous CLL vs. not CLL evaluation have been tested for more than two decades. Many centres still use the scoring system developed by Estella Matutes and colleagues in 1994 [22] but there are issues with reproducibility, and

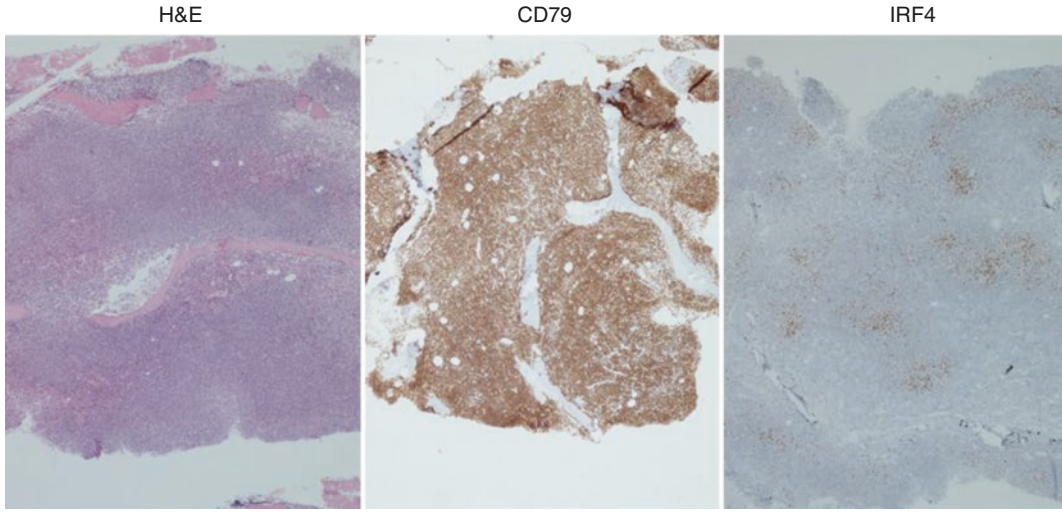


Fig. 2.1 Proliferation centres identified by IRF4/MUM1 expression

giving markers such as CD5 and CD23 equal weighting to other markers is not acceptable in some centres.

2.5.1 CLL Scoring Systems

The initial CLL score was developed by assessment of 666 cases (CLL, 400; prolymphocytic leukaemia, 22; HCL, 40; HCLv, 15; SLVL, 100; FCL, 26; LPL, 25; MCL, 20; and DLB, 18). On the basis of the most common marker profile in CLL (CD5+, CD23+, FMC7−, weak sIg and weak CD22), markers are assigned a value of 1 or 0 according to whether it is typical or atypical for CLL. Scores range from 5 (typical of CLL) to 0 (atypical for CLL) with approximately 10% of cases classified as CLL scoring 3 or below [22]. The scoring system was subsequently revised to replace CD22 with CD79b, which was reported to improve diagnostic accuracy from 91.8% to 96.8% for a score of 3 or higher to differentiate CLL vs. other B-LPD [67], and this algorithm continues to be used in a substantial proportion of diagnostic laboratories. Thomas Köhnke and colleagues [18] recently re-appraised the scoring approach because the previous scores had not evaluated CD200 expression, and their data indicated that dual positivity for CD5 and CD23 showed much higher specificity and sensitivity for the diagnosis of CLL than approaches which consider the marker separately, and the higher reproducibility of CD79b compared to sIgM for discrimination of CLL. Their "CLLflow score" [18] is calculated by adding the percentages of CD200+ and CD23+/CD5+ B-cells and then subtracting the percentages of CD79b+ as well as FMC7+ B-cells, such that if the score is higher than zero, a diagnosis of CLL is likely. The CLLflow score showed comparable sensitivity (97.1%, AUC = 0.98) vs. the modified Matutes score (98.6%) but increased specificity (87.2% vs. 53.8%, $P < 0.001$) [18].

2.5.2 ERIC/ESCCA Approach to Improving Diagnostic Reproducibility

In addition to the fact that many centres require both CD5 and CD23 co-expression to make a diagnosis of CLL, a further potential limitation of the published scoring systems is that diagnostic laboratories use different analysers and reagents and therefore there is no reproducible approach to defining a positive/negative threshold, defined either as 20% or 30% above background or variable according to the assay-dependent limit of blank, and there has been no definition of weak expression [68–71]. Approaches to harmonise results across different instruments have been reported by the Harmonemia group [72], and the Euroflow group has validated a specific reagent set with standardised instrument settings and protocols [14, 73] although cost may be limiting for some diagnostic centres. The ERIC and ESCCA groups have attempted to identify a consensus for reproducible identification of CLL. Markers considered as "required" for the diagnosis of CLL by the participants in this study (CD19, CD5, CD20, CD23, Kappa and Lambda) are consistent with current diagnostic criteria and practice. In addition, a consensus "recommended" panel of markers to refine diagnosis in borderline cases was identified (see Table 2.1), containing CD43, CD79b, CD200 and ROR1 for the reasons discussed above as well as CD81 to facilitate subsequent MRD analysis [41]. Cases not meeting the criteria require a multidisciplinary diagnosis. Importantly, definitions for positive, negative and weak were defined based on measurable differences identified in the International Clinical Cytometry Society (ICCS) and International Council for Standardization in Haematology (ICSH) guidelines [69], and a reproducible approach to validate and apply these markers in individual laboratories was identified [53]. The approach is currently undergoing prospective validation.

Table 2.1 Proposed ERIC/ESCCA required and recommended markers

Inclusion in diagnostic panel	Antigen	Expression in CLL (% pos vs. control)	Control population in normal peripheral blood		Minimum relative fluorescence intensity of positive and negative control populations (preferred)
			Positive	Negative	
Required	CD19	Positive (>95%)	B-cells	T-cells	≥10[a]
	CD5	Positive (>20%)	T-cells	B-cells	≥30 (≥65)
	CD23	Positive (>20%)	B-cells	T-cells	≥5[a]
	CD20	Weak	B-cells	T-cells	≥10 (≥20)
	Igκ/λ	Weak and restricted	B-cells	T-cells	≥5[a]
Recommended	CD43	Positive (>20%)	T-cells	B-cells	≥15 (≥40)
	CD79b	Weak	B-cells	T-cells	≥15 (≥30)
	CD81	Weak	T-cells	Granulocytes	≥12 (≥20)
	CD200	Positive (>20%)	B-cells	T-cells	≥5[a]
	CD10	Negative (<20%)	Granulocytes	T-cells	≥10[a]
	ROR1	Positive (>20%)	B-progenitors	T-cells	≥5[a]

Minimum relativel fluorescence intensity values refer to the relative signal on positive versus negative control populations specifically validated to achieve optimal separation of CLL cells from normal B-cells except markers denoteed [41]

[a]which are consensus values [53]

2.6 Molecular Diagnostics

There is no pathognomonic molecular abnormality in CLL. The most common abnormalities are chromosomal copy number abnormalities, including focal deletions of 13q, 11q or 17p, and trisomy 12. Whole exome/genome studies have also identified >40 driver mutations, with the most common being SF3B1, ATM, NOTCH1 and TP53. Common driver mutations are frequently encoded in the minimally deleted regions, including ATM and BIRC3 within 11q, TP53 within 17p and mir15a/16-1 within 13q. Translocations are infrequent, and nearly 10% of patients have no detectable molecular abnormality [7, 74, 75].

2.6.1 Chromosomal Deletions and Aneuploidy

13q14 is the most frequently deleted region in CLL and contains two microRNA genes mir-15a and mi-r16-1 within a 30-kb region between exons 2 and 5 of the DLEU2 gene [76]. Mir15/16 negatively regulates Bcl2 at a post-transcriptional level, and the deletion results in overexpression of BCL2 [77]. Abnormalities are not restricted to the 30-kb region, with larger deletions associated with increased probability of disease progression [78]. Deletion of 13q14 is reported to be an early or founder genomic lesion [79] and is sufficient to cause development of indolent B-lymphoproliferative disorders in mice that recapitulate the spectrum of CLL-associated phenotypes observed in humans [80]. Deletions of 13q are common in a variety of different disorders, including approximately 40% of mantle cell lymphoma cases [81], and therefore identification of a 13q14 deletion does not necessarily indicate a diagnosis of CLL. However, in a CD5+ B-lymphoproliferative disorder with no evidence of a *t*(11;14), the detection of 13q14 deletion may be supportive of a diagnosis of CLL.*Trisomy 12* is detected in 10–20% of CLL but is also detectable in other B-cell disorders including 17% of mantle cell lymphoma [81] and 4% of Waldenstrom macroglobulinemia [82]. There is a close association between trisomy 12 and NOTCH1 mutations [83, 84]. Trisomy 12 is associated with atypically strong CD20/FMC7 and sIg/CD79b as well as atypical morphology [85–87] and does not appear to be an adverse prognostic factor in the absence of NOTCH1 mutation [88]. Although trisomy 12 is not specific for CLL, in the context of a CD5+CD23+ B-lymphoproliferative disorder that is phenotypically and/or morphologically atypical for CLL the detection of trisomy 12 as a sole abnormality would be consistent with a diagnosis of CLL.

2.6.2 Chromosomal Translocations

The translocation of CyclinD1 (BCL1 or CCND1) to IGH was originally reported as an infrequent abnormality of atypical CLL or other lymphomas that were later re-classified when the *t*(11;14) was identified as a characteristic of MCL [89]. CCND1–IGH translocations are not reported in CLL [74] because the translocation would automatically result in diagnosis of MCL, but it is less clear how frequently a CCND1–IGH translocation is detectable in cells with a typical CLL phenotype. This is important because in resource-limited settings, some centres do not perform cytogenetic analysis in cases with a typical CLL phenotype that does not require immediate treatment. In a series of 1032 patients with a presumptive diagnosis of CLL assessed for an IGH translocation, 10/1032 (1%) had a CCND1–IGH fusion. The translocation was not detected in any cases with a CD5/CD20/CD23 expression typical for CLL, although one of the patients had both CLL-phenotype and MCL-phenotype (CD5+CD20++CD23−) monoclonal B-cells present with a CCND1–IGH fusion detected only in the MCL-phenotype cells [90]. To date in our centre, the *t*(11;14) translocation has not been identified in any CD5+CD23+CD200+ B-LPD with a fully typical phenotype (including analysis of CD20, CD43, CD79b, CD81 and ROR1, $n > 300$) [35]. BCL2 and BCL3 translocations are relatively rare events in typical B-CLL, detected in ~1–4% of cases. The BCL2 rearrangements involve hot spots of recombination distinct from those commonly seen in lymphoma, suggesting an alternative pathogenic mechanism. They are frequently associated with 13q deletion or trisomy 12, and there is no evidence that BCL2 or BCL3 translocation impact on prognosis [74, 90–92].

2.6.3 The Immunoglobulin Gene

The immunoglobulin gene repertoire in CLL is markedly skewed relative to normal B-cells, and approximately half of CLL are somatically hypermutated (>2% difference from germline). CLL patients with IGHV-germline/unmutated disease had an increased risk of progression with poorer survival compared to patients with IGHV-mutated CLL [93, 94]. IGHV sequence analysis in both Europe and the USA led to the identification of subsets of cases carrying highly similar BCR Igs among both mutated and unmutated cases, termed stereotyped BCR [95]. The specific stereotype may modulate the impact of other molecular abnormalities [96]. Furthermore, the IGHV mutation status is reported to be associated with outcome depending on treatment type, with IGHV-mutated cases achieving improved outcomes with chemoimmunotherapy [97–99]. The therapeutic efficacy of inhibiting B-cell receptor signalling [100, 101] and the evidence for cell-autonomous B-cell receptor (BCR)-mediated signalling in CLL [102] demonstrate the significance of the immunoglobulin gene in the pathogenesis of CLL. It is also notable that the immunoglobulin gene repertoire of MBL with lymphocytosis is similar to CLL, whereas the very-low-level CLL-phenotype monoclonal B-cells that are frequent in the general population and show no evidence of disease progression have a different immunoglobulin gene repertoire to CLL [103]. Although the immunoglobulin sequence currently does not contribute directly to diagnosis in CLL, it is an important analysis because the mutation status (and stereotype subset if identifiable) is a powerful prognostic factor that may impact on the approach to treatment.

2.6.4 Other Molecular Abnormalities

Abnormalities with prognostic implications but not contributing to differential diagnosis include:

17p deletion and/or TP53 mutation, 11q deletion and/or ATM mutation and BIRC3 and SF3B1 mutations. These molecular abnormalities are discussed in Chap. 4, “Prognostics Markers”.

2.7 Differential Diagnosis

In the majority of new cases, the diagnosis of CLL is very straightforward based on peripheral blood features alone because most patients

present with a mild lymphocytosis showing a completely typical morphology and immunophenotype without other symptoms. In cases with cytopenia, laboratory investigations and/or bone marrow biopsy to exclude extensive infiltration are sufficient to identify the appropriate clinical management. In cases with a low level of peripheral blood involvement with CLL-phenotype monoclonal B-cells but with lymphadenopathy/splenomegaly, a tissue biopsy will be required to exclude lymphoma with co-incidental MBL/early stage CLL. However, the diagnosis for cases with a CD5+ monoclonal B-cell expansion that does not have the typical phenotype for CLL may be more challenging because there is substantial and increasing overlap in the immunophenotype and molecular abnormalities identifiable in current diagnostic categories.

2.7.1 Mantle Cell Lymphoma

The differential diagnosis between CLL and MCL is determined in the vast majority of cases by FISH analysis to assess the presence of a CCND1–IGH translocation. However, in some cases a diagnosis of MCL may be made on the basis of a CCND2 translocation or SOX11 expression [4, 104]. Difficulties in diagnosis may arise in peripheral blood or bone marrow aspirate samples demonstrating a CD5+ monoclonal B-cell expansion with a phenotype that is otherwise atypical for CLL but without access to diagnostic material/tests for aberrant SOX11 or Cyclin D2/3 expression. Caution is required in cases with weak or absent CD23 and/or CD200 expression, particularly if there is also moderate to strong CD38 expression, and weak or absent CD43/ROR1. The detection of trisomy 12 or deletion 13q14 does not exclude a diagnosis of mantle cell lymphoma. In many cases, the necessary diagnostic material may not be taken because treatment is not indicated. Active monitoring may be equally appropriate for SOX11-negative low-level leukaemic/non-nodal MCL or early stage CLL. If there is an indication for treatment, a biopsy would be required for immunohistochemistry and/or to obtain sufficiently involved material to perform FISH analysis.

2.7.2 B-Prolymphocytic Leukaemia

B-PLL as a distinct diagnostic entity is difficult to define due to a high degree of overlap with MCL or DLBL. B-PLL is defined as detection of >55% prolymphocytes in microscopy. Reported series indicate a high proportion of TP53 abnormalities [105]. Gene-expression profiling distinguishes between cases identified as B-PLL vs. CLL or SMZL but demonstrates overlap with MCL [106]. The protein expression profile is reported to indicate that B-PLL represents a subset of MCL, irrespective of the presence or absence of *t*(11;14) [107].

2.7.3 Waldenström's Macroglobulinemia/Lymphoplasmacytic Lymphoma

In most cases, WM/LPL is distinct from CLL with respect to morphology, phenotype and molecular features. However, CLL and WM/LPL have a similar protein expression profile with respect to several markers, including CD22 and CD25 expression, and the differential diagnosis may be difficult in some WM/LPL cases with limited plasma cell differentiation and CD5 expression on the B-lymphocyte compartment. Although there is overlap in the treatment approaches for WM/LPL and CLL, it is important to note that BTK inhibition has very limited efficacy in WM cases that lack a MYD88 mutation [108], i.e. a CD5+ WM/LPL with wild-type MYD88 may respond sub-optimally to BTK inhibition. A CD5+ B-lymphoproliferative without ROR1/CD43 expression, and with a MYD88 mutation, or lack of IRF4+ proliferation centres,

or increased numbers of mast cells may be more likely to represent a subset of WM/LPL than CLL, and the diagnosis and treatment approach should be made with caution.

2.7.4 Cold Agglutinin Disease (CAD)

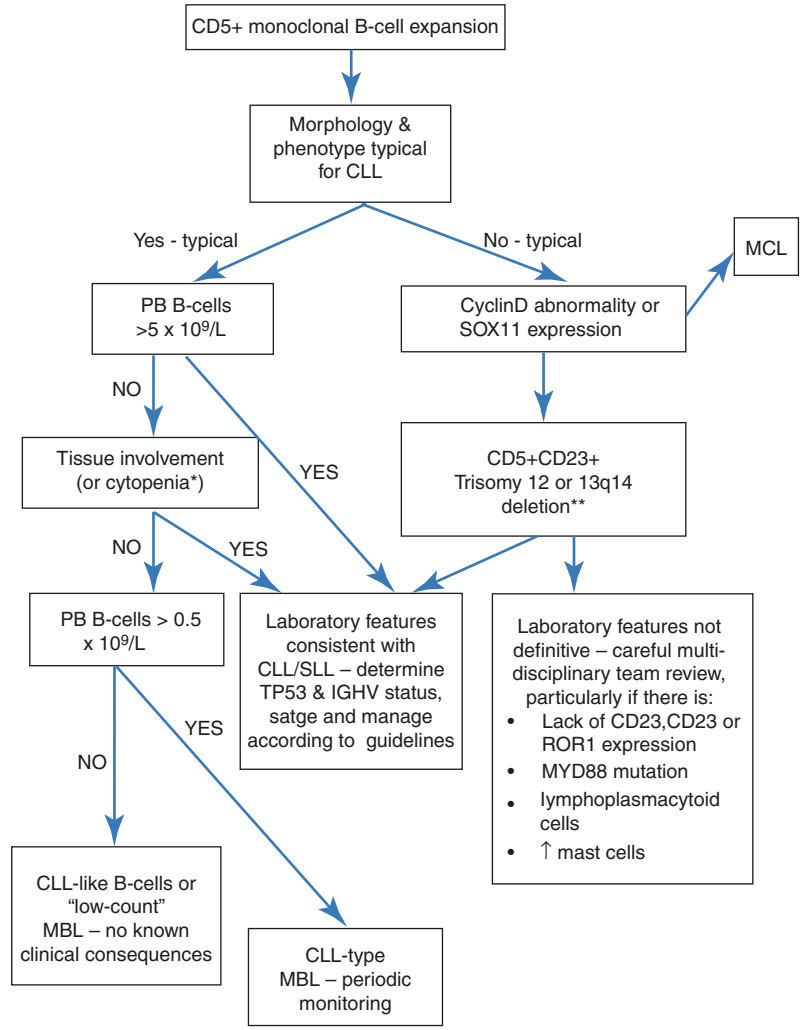
Primary CAD is a haemolytic disease mediated by monoclonal IgM autoantibody with a predominant specificity for the blood group antigen i/I and encoded by the IGHV4-34 immunoglobulin heavy chain gene [109]. Although often considered as a sub-category of WM/LPL, the monoclonal B-cells in CAD are phenotypically distinct with CD5 expression in >80% of cases in our series, and 60% co-expressing CD23. The monoclonal B-cells in CAD show stronger CD20/CD22/CD81 and CD79b/sIg than is typical for CLL, and also co-express CD95. Plasmacytoid differentiation is less frequent than in WM/LPL and the MYD88 L265P mutation is infrequent in CAD [110, 111] indicating that this disorder is distinct from both CLL and WM/LPL.

2.7.5 Monoclonal B-Cell Lymphocytosis

MBL with CD5 expression and weak CD20 (CLL-like MBL) shows a protein expression profile that is almost identical to CLL for a large range of markers, with the exception of CD38/CD49d expression which is reduced in MBL compared to CLL consistent with other prognostic features [112]. In the absence of cytopenia or organomegaly, the differential diagnosis according to the revised WHO criteria rests solely on the absolute B-cell count [4] although IWCLL guidelines indicate that MBL with cytopenia should still be considered as CLL [1]. The presence of low-level lymph node or bone marrow involvement may be expected in CLL-type MBL and should not necessarily change the diagnosis to CLL [4] or lead to initiation of treatment in the absence of other evidence of progressive disease. Identifying the clinical relevance of non-CLL-phenotype monoclonal B-cell expansions may be particularly challenging because there may be substantial bone marrow disease in cases with low-level peripheral blood involvement.

2.8 Richter's Syndrome/Large Cell Transformation

Richter's syndrome (RS) is defined as the development of diffuse large B-cell lymphoma in patients with a previous or concomitant diagnosis of CLL [4]. RS occurs in 2–3% of patients at a rate of ~1% per year for previously treated patients and ~0.5% per year for untreated patients [113]. Approximately, 80% of RS cases are clonally related to the underlying CLL, while 20% have distinct IGHV-D-J rearrangements and represent de novo DLBL in a CLL patient [114]. RS is not usually present in all lymph node sites, and the lesion displaying the largest diameter by imaging, the most rapid kinetics of progression and/or the most FDG avid at 18FDG PET/CT should be biopsied [114]. RS requires morphological demonstration of confluent sheets of large neoplastic B-cells, with the majority of cases showing loss of CD5 and/or CD23 expression [115]. RS that is clonally unrelated to the underlying CLL typically has a lower frequency of TP53 abnormalities and an outcome similar to de novo DLBL and it is debatable whether such cases should be called RS for the purpose of clinical trials [116]. Clonally related RS has a much poorer outcome and the diagnostic laboratory should focus on distinguishing clonally related large cell transformation from clonally unrelated DLBL and progressive CLL. The clinical and biological aspects of RS are detailed in Chap. 10.



* WHO 2016 indicate that cytopenia considered separately, IWCLL guidelines indicate cytopenia requires diagnosis of CLL

** in the absence of a molecular abnormality diagnostic of another B-LPD

2.9 Summary

Although there remains no pathognomonic genetic lesion or gold standard for CLL diagnosis, there is a characteristic set of morphological, immunophenotypic and genetic features that permits straightforward diagnosis in the majority of cases. Collaborative efforts to improve the reproducibility of diagnosis are underway and in addition to CD19, CD5, CD23, CD20 and Igκ/λ/CD79b, it is recommended to evaluate CD43, CD200 and ROR1 to facilitate distinction of CLL from MCL and WM/LPL/MZL. It is essential that cytopenias are fully investigated in patients with MBL or low-level CLL because CLL-like monoclonal B-cell expansions are highly prevalent in the general population and are usually not associated with cytopenia. Sequence analysis is increasingly involved in the diagnosis and optimisation of treatment in CLL. The expanding knowledge of driver mutations across different B-cell malignancies is facilitating differential diagnosis in cases with intermediate laboratory features. For patients with progressive disease, the outcomes

are improving with strategies to counteract TP53 and ATM abnormalities. The immunoglobulin gene is central to the pathogenesis of CLL, and sequence analysis is important not just for prognosis but also potentially to optimise treatment and distinguish Richter's syndrome from de novo DLBL. As therapeutic pathways and combinations are identified, the laboratory diagnosis of CLL is continuing to evolve in order to enable patients to be assigned the optimal treatment.

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Part III

Clinical Presentation

The Clinical Presentation of CLL

3

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3.1 Introduction

CLL is predominantly a disease of the elderly and has a variable clinical presentation and subsequent evolution. Clinical features and laboratory investigations are important for making decisions about patient management and for predicting outcomes, which are very variable in this disease.

There has been a lot of progress in the last decade in our understanding of the factors that determine the clinical evolution of CLL as well as its pathogenesis and molecular genetics. Still, patient-related criteria, such as symptoms and physical signs and simple blood tests, are the backbone for the clinical staging and management planning.

3.2 Demographics

3.2.1 Incidence

The incidence of CLL in the USA and Western Europe is between 4 and 5 per 100,000 persons per year equating to a lifetime risk of developing CLL of 0.6 in the USA (2011–2013 data) [1] and 1/155 men and 1/260 women in England (2013–2014 data) [2]. There are marked racial differences in the incidence of CLL; it is five- to tenfold lower in Asians compared to those of European descent [3].

Epidemiological surveys have shown that CLL has one of the highest familial risks of any cancer, with an 8.5-fold increased risk among first-degree relatives of developing CLL and a 1.9-fold risk of developing other B-cell chronic lymphoproliferative disorders, especially lymphoplasmacytic lymphoma and hairy cell leukaemia [4, 5]. Genome-wide association studies have identified over 30 single nucleotide polymorphisms (SNPs) mapping in, or close to, genes with roles in B-cell biology [6].

3.2.2 Age Distribution

There is a slight discrepancy in the literature concerning the median age of CLL patients at diagnosis. In most registries, this is between 70 and 72 years [1]. In patients entered into clinical trials, the median age is lower. This may be because few elderly patients have been entered into treatment trials in the past, often due to exclusion criteria which debar patients who are older or who have conditions which are commoner in the elderly, such as organ dysfunction or other malignancies. Recent trials cater specifically for more elderly patients with the use of less

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toxic oral agents, obviating the need for multiple hospital visits and in-patient care. Other reasons for the younger age of patients entered into trials may be that disease requiring treatment is diagnosed earlier than more benign disease, or that CLL may have a more benign clinical course in the elderly, resulting in later diagnosis. In studies running in the UK between 1979 and 2004, comprising a total of 3120 patients, the median age of patients randomised into treatment trials was 65 years, whilst in those entered into observational studies of stage A patients the median was 67 years. The age distribution of these two groups is shown in Fig. 3.1 where it can be seen that the observational studies had a higher proportion of patients in the older age bands.

Similarly, the median age of the 3472 patients included in the CLL International Prognostic Index (CLL-IPI) [7] was 61 years, because the majority derived from randomised trials, whilst in the Danish National CLL registry, in which 80% were Binet Stage A patients, the median age was 70 years [8]. Only 7% of patients in the UK clinical trials depicted in Fig. 3.1 were aged <50 years, the youngest patients being 31 years old.

The relevance of age is that it remains an important predictor of overall survival (OS), as illustrated in Fig. 3.2. It is one of the five independently significant variables contributing to the CLL-IPI prognostic index [7].

3.2.3 Gender

It has long been recognised that twice as many men as women develop CLL, with a male:female ratio of 2:1. However, data from the UK trials [9] and a review of the literature show that the male:female ratio varies with the stage of the disease. In the condition preceding CLL, known as monoclonal B-cell lymphocytosis, the male:female ratio is 1:1. The ratio increases in the early stages of the disease, namely Rai Stage 0 and Binet Stage A, and increases again above 2:1 in patients needing treatment (Table 3.1).

Confirmation of the evidence that men more frequently develop progressive CLL comes from an analysis of cases included in the CLL-IPI study [7]. When patients from two large data sets are distributed according to the four CLL-IPI risk categories, the male:female ratio increases threefold in the higher risk cases (Table 3.2). This supports the concept that women have a more benign form of CLL and respond better to therapy, including chemoimmunotherapy, than men [9, 10]. The corollary is that CLL in men runs a more aggressive course. Our evidence showed that there are several reasons for this difference, of which the most important is perhaps the prevalence of biological markers of good prognosis in women [9]. As a result, the OS in all the UK CLL trials was better in women than in men, as also was progression-free survival (PFS), which was measured in the LRF CLL4 trial [9]. These results were confirmed in the German data derived from chemoimmunotherapy trials [10]. In the CLL-IPI study, women had a better median OS than men, 124 vs. 84 months respectively, with the proportion surviving at 5 and 10 years being significantly better ($p < 0.0001$) [7].

3.3 Clinical Features

3.3.1 Presentation

The commonest presenting features of CLL are fatigue, infections, particularly bacterial infections of the respiratory tract, and lymphadenopathy. Other symptoms may be involuntary weight loss or unexplained fever. The incidence of these features has fallen in recent decades and over 80% of cases are now diagnosed with early asymptomatic disease based on the finding of a lymphocytosis in a blood count performed for an incidental reason [11]. Such asymptomatic cases have early CLL (Rai Stage 0 or Binet Stage A) and only need to be followed for the first few months to confirm the diagnosis, ascertain features of progression and decide on the clinical staging (see below). Features that become more frequent during the course of the disease, and especially following treatment, include opportunistic infections, psychological problems and second malignancies.

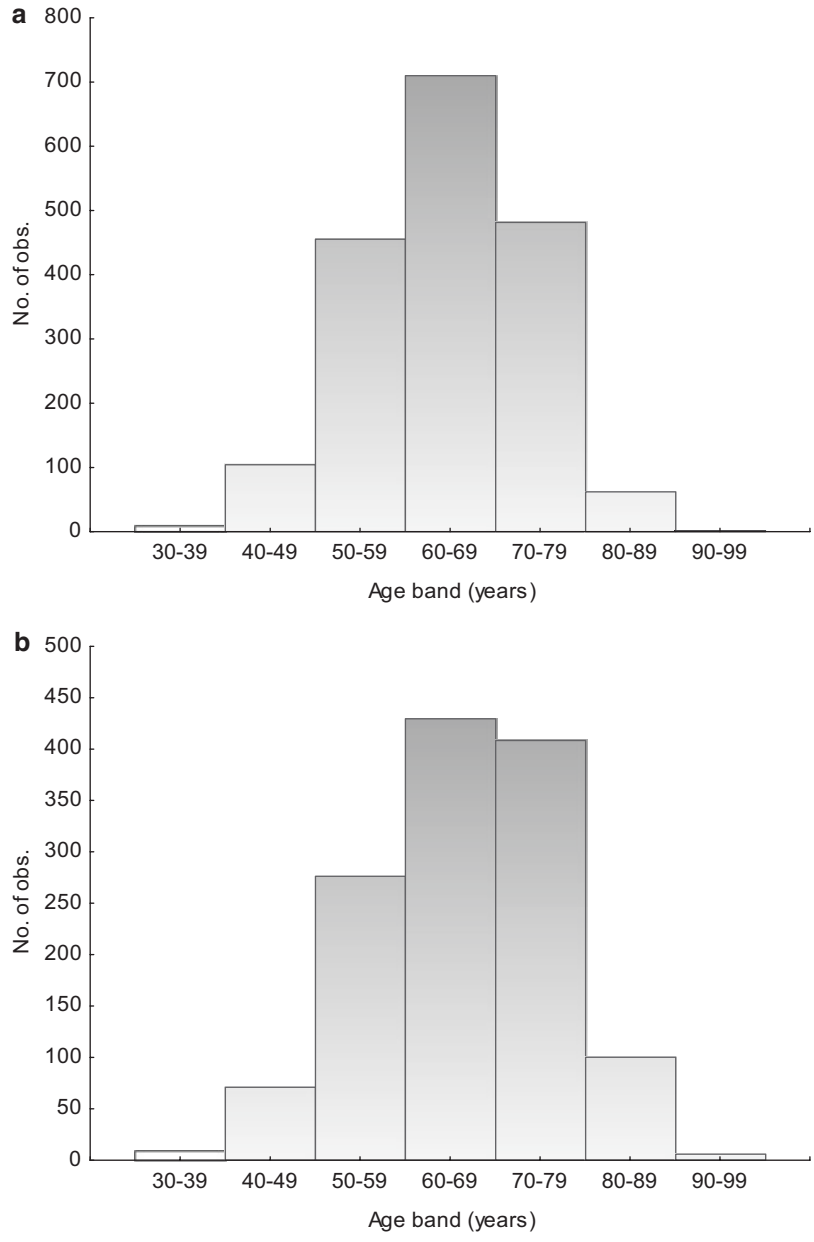

Fig. 3.1 UK CLL trials: histograms showing the age bands of patients at study entry. (**a**) Randomised patients in the UK CLL trials 1–4 (n = 1821). (**b**) Registration-only patients (observational studies) (n = 1299)

Clinical features are often classified based on whether they are patient-, disease- or treatment-related, and whether disease-related features reflect tumour burden or the immune dysfunction that accompanies CLL. In practice, many symptoms have a number of possible causes and, in an individual patient, are often multifactorial. For example, fatigue may be a consequence of increased cytokine production, anaemia caused by marrow suppression or red cell autoantibodies, or depression.

For patients, particularly the elderly, who are considered for treatment and/or entry into clinical trials, it is always important to assess comorbidities and performance status, as a higher disease burden and greater number of comorbidities correlate with a worse OS [12] (see also Chap. 6).

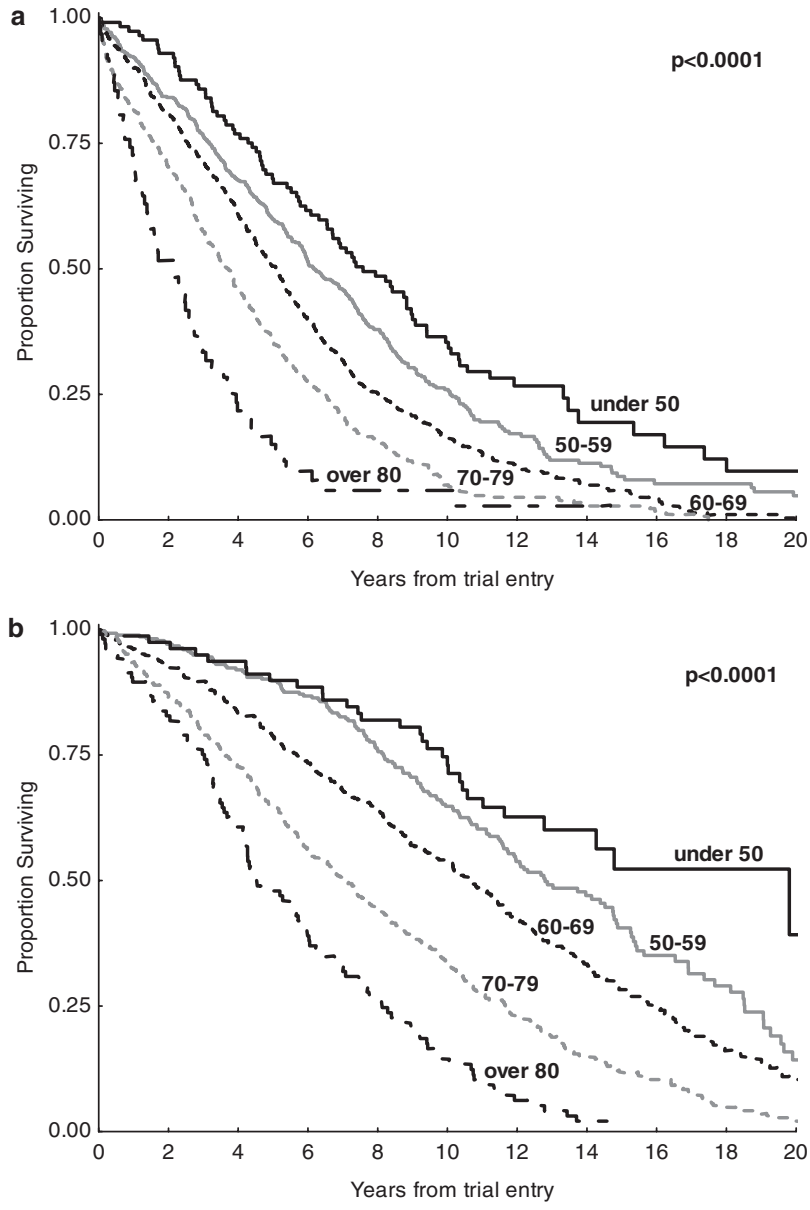

Fig. 3.2 UK CLL trials: overall survival from trial entry by age band (years). (**a**) Randomised patients in the UK CLL trials 1–4 ($n = 1821$). (**b**) Registration-only patients (observational studies) ($n = 1299$)

Table 3.1 Male:female ratios according to clinical status

	No. of patients	Male:female ratio
Monoclonal B-cell lymphocytosis [9]	996	1.1: 1
Stage A/0—literature [9]	1816	1.3: 1
Stage A—UK trials [9]	1299	1.5: 1
Danish national registry [8][a]	3023	1.5: 1
Clinical trials—literature [9]	2399	2.7: 1
UK randomised trials [9]	1821	2.7: 1
German CLL study group [10][b]	1078	2.7: 1

[a]This study applied the International Prognostic Index (CLL-IPI) in a population-based cohort. The majority (79%) were stage A

[b]Chemoimmunotherapy trials

Table 3.2 Male:female ratios according to the CLL-IPI[a]

Risk groups (score)	Main data set $N = 1799$	Training data set $N = 1214$
Low risk (0–1)	1.74	1.56
Intermediate risk (2–3)	2.37	2.22
High risk (4–6)	3.03	3.16
Very high risk (7–10)	4.43	3.77

[a]J Bahlo, personal communication of data from patients included in a study by the CLL International Prognostic Index (CLL-IPI) working group (2016) [7]

3.3.2 Lymphoid Involvement

Enlarged lymph nodes, usually >1 cm in diameter, are painless, largely symmetrical and may involve the neck (anterior or posterior triangle and/or supraclavicular region), axillae and the inguinal region, including superficial femoral nodes. Lymphadenopathy in these three areas is used for staging purposes together with splenomegaly and hepatomegaly [13, 14]. It is important to be aware that an enlarged liver may have other causes such as congestive heart failure. A palpable spleen below the left costal margin is often associated with palpable nodes but in <5% of cases may be the only physical finding. Abdominal fullness and/or discomfort in the left hypocondrium may indicate splenomegaly. In younger male patients, significantly enlarged nodes may be associated with 11q deletion by cytogenetic (fluorescence in situ hybridisation—FISH) analysis.

3.3.3 Extramedullary Features

Clinical and/or laboratory abnormalities of many non-haematological organs or systems such as neurological symptoms or renal dysfunction are common in CLL. These may be related to infections, autoimmune or inflammatory disorders, treatment toxicity or unrelated morbidities, but another important cause, which it is important not to overlook, is extramedullary involvement by CLL. A Medline search of cases reported between 1975 and 2012 identified 192 such cases [15]. The most commonly reported sites were: skin (33%), central nervous system (CNS) (27%), gastrointestinal (GI) tract (14%), genito-urinary/gynaecological (10%), lung (5%) and ocular (5%). Survival from the diagnosis of extramedullary disease varied with the site of involvement and was worst for CNS disease. Diagnosis may be straightforward if there is a solid mass to biopsy, but can be more difficult if there is diffuse tissue infiltration with small lymphocytes. The latter is a common post-mortem finding in the absence of clinically significant ante-mortem organ dysfunction. Similarly, the sensitivity of cerebrospinal fluid (CSF) analysis to detect CNS involvement by CLL in a single centre study was 89% but the specificity was only 42%, reflecting the frequency of CLL cells in the CSF in other neurological conditions affecting patients with CLL [16]. The incidence of extramedullary disease is difficult to ascertain as it is frequently reported in the form of single case reports or small series. The German CLL Study Group analysed the disease status of patients at the time of entry into three first-line chemo or chemoimmunotherapy trials [17]. Extramedullary disease, excluding CNS involvement, was found in 3.6% of patients with the commonest sites being lung/pleural effusion, GI tract and skin.

A physical examination of the patient should include careful examination of the skin for signs of pallor, purpura or skin lumps representing CLL infiltration, although these are uncommon at clinical presentation.

3.3.4 Constitutional Symptoms

Symptoms such as fatigue, night sweats, weight loss, disturbed sleep and low grade fever in the absence of an alternative cause are features of progressive CLL, and their sudden onset in conjunction with rapidly enlarging lymph nodes may signal disease transformation. Indications for treatment in the current IWCLL guidelines [18] include severe and persistent constitutional symptoms, but a minority of mostly early stage patients with less severe symptoms may nevertheless suffer an impaired quality of life. Based on the association between constitutional symptoms and raised levels of circulating cytokines, and the finding of activated *JAK2/STAT3* signalling in CLL, a phase II trial of the *JAK1/JAK2* inhibitor, ruxolitinib, in patients with no

indication for systemic CLL therapy, showed a reduction in constitutional symptoms in the majority of patients [19].

3.3.5 Second Malignancies

Since the 1970s, numerous studies have documented an increased risk of second malignancies in patients with CLL compared to an age-matched general population and have speculated on the role of disease- or therapy-related immunosuppression and therapy-related carcinogenesis as contributory factors. Standardised incidence ratios for all second malignancies, and for specific solid tumours, primary haematological malignancies and Richter's transformation, vary among series, reflecting differences in demographics, treatment exposure and duration of follow-up [20–22]. The increasing incidence of second tumours as a cause of death in CLL, and the negative impact of CLL on the management of some second tumours, highlights the importance of identifying and screening those CLL patients most at risk of second tumours. Risk factors for solid and haematological malignancies in patients treated with first-line alkylating agents, purine analogues and/or rituximab include age, male gender, comorbidities and at least one subsequent treatment. Risk factors for skin cancers are a prior history of skin cancer and "poor risk" CLL at diagnosis, as defined by the CLL-IPI index [22–24].

3.4 Laboratory Investigations

3.4.1 Full Blood Count

The main finding of the initial tests is evidence of lymphocytosis with at least 5×10^9/L clonal B-cells by light chain restriction (either kappa or lambda) required for diagnosis [18].

CLL lymphocytes are small, with a rim of cytoplasm and a characteristic clumped nuclear chromatin without a visible nucleolus. Smear cells, or Gumprecht nuclear shadows, in blood films are also a typical feature of CLL and not seen in other disorders evolving with lymphocytosis. Larger cells with a prominent nucleolus (prolymphocytes) are always seen in blood films, usually <5% (Fig. 3.3).

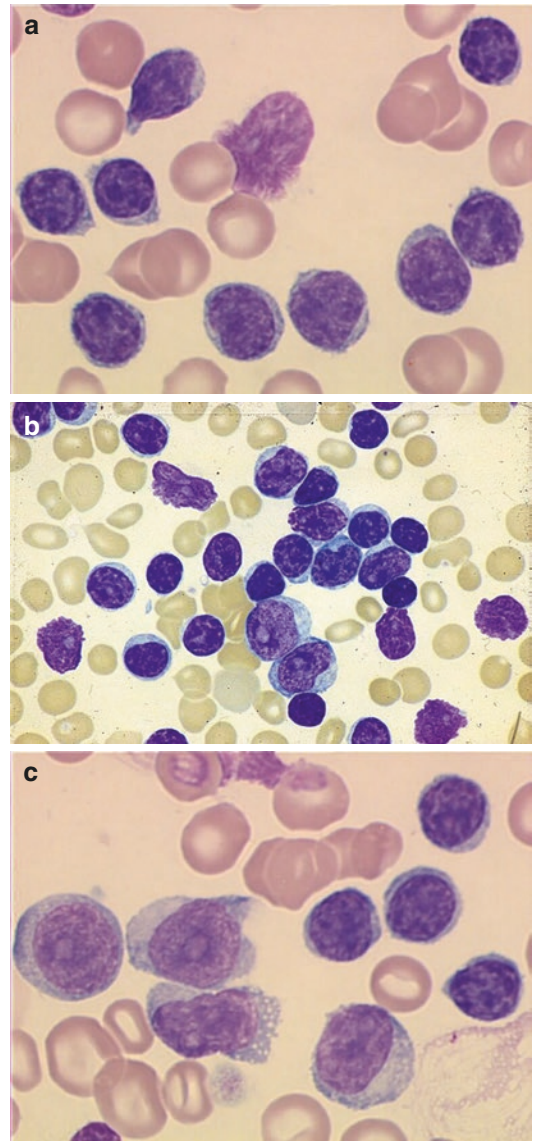

Fig. 3.3 CLL blood films. (**a**) Typical CLL. All the cells are small lymphocytes and there is a single smear cell (reproduced from Oscier et al. 2016 [25], courtesy of BJH). (**b**) Typical CLL with mostly small lymphocytes; there are two prolymphocytes in the lower half and a few smear cells. (**c**) Typical CLL/PL (defined as CLL cases with 11–55% circulating prolymphocytes). There is a mixture of prolymphocytes and typical CLL lymphocytes (reproduced from Oscier et al. 2016 [25], courtesy of BJH)

In an analysis of the morphology of peripheral blood films of over 500 patients entered in the LRF CLL4 trial, the majority (86%) had <10% prolymphocytes [25]. The finding of more than 10% prolymphocytes in the remaining 14% of patients was associated with a shorter PFS and OS and correlated with the presence of *NOTCH1* mutations, absence of 13q deletions, higher CD38 expression and unmutated *IGHV* genes, all associated with poor prognosis [25]. Therefore, a careful examination of blood films is important to trigger other investigations and to evaluate features of clinical progression.

Two other features of the blood count, the haemoglobin level and the platelet count, are necessary to define cytopenias and are an integral part of the staging systems [13, 14] (see Sect. 3.5). A raised reticulocyte count together with anaemia is suggestive of autoimmune haemolytic anaemia (AHA), whilst a very low or absent reticulocyte count together with anaemia suggests pure red cell aplasia (PRCA).

Lymphocyte counts are one of the best indicators of disease progression. It is important to assess the lymphocyte doubling time (LDT) in asymptomatic patients presenting in early stage CLL with no other features of disease progression. Lymphocyte counts need to be repeated initially every 2–4 weeks to calculate the LDT. The counts can be plotted in a semi-logarithmic chart to document an exponential increase as a straight line or can be determined by linear regression extrapolation to calculate the LDT. An LDT of <12 months has been considered evidence of progression, though the IWCLL guidelines have now recommended a shorter period of 6 months. When the lymphocyte count is artificially raised by factors such as infections, or treatment with corticosteroids or the new kinase small molecule inhibitors, this is not considered as evidence of progression.

3.4.2 Other Blood Tests

Biochemical screening is necessary to assess renal and liver function prior to therapy and as part of the total clinical evaluation on presentation.

Beta-2 microglobulin (B2M), a single chain part of the major histocompatibility complex class I proteins, has become an important prognostic marker associated independently with PFS and OS. A level >3.5 mg/L is associated with poor prognosis in the CLL-IPI risk assessment [7]. B2M is cleared by glomerular filtration and catabolised in the proximal renal tubule and therefore it also increases when the renal function is impaired. See also Chap. 4.

Serum lactate dehydrogenase (LDH), a marker of cell turnover, also correlates with disease activity and worse prognosis. In the CLL-IPI study, half the cases had levels >250 U/L and, in univariate analysis, they had a worse outcome than those with levels below 250 U/L, with a median OS of 80 and 124 months, respectively ($p < 0.0001$). However, in multivariate analysis, LDH did not qualify as an independent factor in the CLL-IPI prognostic index [7]. Another recent report described an association between LDH and PFS in patients with trisomy 12. One third of the 222 patients tested had LDH levels above normal and had a significantly shorter PFS than those with normal LDH [26]. High levels of both LDH and B2M have also been reported in cases with expanded proliferation centres in tissue biopsies and progressive CLL [27].

Serum immunoglobulins (Igs) are generally decreased in CLL, and the low levels may be largely responsible for the high frequency of respiratory infections. Serum Igs tend to decrease with disease progression. Small monoclonal bands, usually IgM, are seen in c.10% of cases. There is no evidence that the serum Igs or the monoclonal bands have prognostic connotations.

A direct antiglobulin test (DAT), or Coombs test, is a useful baseline investigation as it may predict the development of AHA after treatment. In the LRF CLL4 trial, 14% of patients had a positive DAT test at entry and this correctly predicted the development of AHA in one out of three patients, whilst a DAT negative test was >90% correct in predicting that patients would not subsequently develop AHA. The incidence of AHA in that trial was 10%, similar to the incidence reported in the literature. DAT positivity in that trial correlated with Binet stage C and a

higher B2M at presentation [28]. After therapy, there was a higher incidence of AHA when chlorambucil (12%) or fludarabine alone (11%) was given than when fludarabine was combined with cyclophosphamide (5%). This confirms that the more effective treatments may reduce the incidence of AHA, as is also seen when anti-CD20 monoclonal antibodies are used. In the German CLL11 trial, there was a clear trend towards a lower incidence of AHA after treatment with chlorambucil combined with either rituximab or obinutuzumab compared with chlorambucil alone (GCLLSG, personal communication). The use of the BTK inhibitor ibrutinib may also significantly reduce the risk of secondary AHA [29]. In fact, when ibrutinib in combination with immunosuppressive therapy is given to patients with active AHA, this may result in the eventual resolution of this complication. For more details, see also Chap. 9.

3.4.3 Bone Marrow (BM) Examination

Although a BM examination is not a diagnostic requirement [18], it is an important baseline investigation for patients who are considered for treatment. More than 30% of a BM aspirate consists of lymphocytes. A trephine biopsy is more useful than an aspirate to assess cellularity and to identify patterns of infiltration, which tend to correlate with disease burden. Biopsy patterns are interstitial, nodular, mixed nodular and interstitial, or diffuse. The latter reflects heavy BM infiltration with no fatty spaces and correlates with cytopenias and disease burden. The value of a BM test at presentation is fourfold:

1. To assess the nature or cause of cytopenias, particularly in Binet Stage C (or Rai III-IV), for example by showing abundant megakaryocytes in immune thrombocytopenic purpura (ITP), or absence of erythroid precursors in PRCA, complicating the CLL;
2. To help distinguish CLL from other B lymphoproliferative disorders in difficult cases, for example by identifying paratrabecular deposits which are characteristic of follicular lymphoma, or by showing proliferation centres which are unique to CLL;
3. To serve as a baseline from which to assess treatment response. A BM biopsy is essential to document complete remission (CR) [18];
4. To assess prognosis according to the pattern of infiltration described above.

3.4.4 Imaging Tests

A routine chest X-ray is often performed at presentation. It is a useful baseline measure and it may detect pre-existing lung pathology or hilar lymphadenopathy.

Computed tomography (CT) scans of abdomen and pelvis are important to detect enlarged para-aortic nodes and other organ enlargement. Although this information is not required for staging, it is a necessary comparator for later treatment follow-up.

A Spanish group performed routine abdominal CT scans in 140 patients presenting with Rai Stage 0 and found detectable enlarged lymph nodes in 27% [30]. This finding correlated with a greater degree of BM infiltration, shorter LDT and a shorter time to progression and to the need for treatment than in those with a normal CT scan. There was no difference in OS between the two groups. The IWCLL Guidelines do not recommend routine CT scans in patients with Binet stage A or Rai stage 0 as it is important to avoid unnecessary radiation exposure. It can be argued that clinical progression can be detected by other means. Nevertheless, the IWCLL guidelines suggest that clinical studies evaluating the use of CT scans in CLL should be encouraged [18]. The result of a CT scan does not alter staging but it is required as a baseline for patients entered into treatment trials.

Abdominal ultrasounds are less invasive but less useful for detecting abdominal nodes. They may however be used to give a precise measure of the size of the spleen and liver, particularly if enlargement is palpable during physical examination.

3.4.5 Cytogenetics/Molecular Investigations

Tests may be carried out to determine *IGHV* mutation status and to detect cytogenetic abnormalities such as *TP53* deletion/mutation, 11q deletion and trisomy 12. Whilst the results may help to predict the clinical course, it should be emphasised that the indication for treatment does not depend on any of these tests [18]. These important prognostic factors are discussed more fully in Chap. 4.

3.5 Clinical Staging

There are two historical but still highly relevant staging systems for CLL, that of Rai [13] and Binet [14], which have been used for several decades. They are simple to use and rely only on the full blood count and physical examination.

After making a diagnosis, the first task of a physician is to decide on the patient's disease stage. This will guide the initial action plan and further investigations.

There are subtle differences between the Rai and Binet staging, even though the original Rai staging has now been simplified from 5 to 3 risk groups [31] (Table 3.3). One difference is the threshold for the haemoglobin level which defines the more advanced cases: 110 g/L in Rai (high risk, formerly III) and 100 g/L for Binet stage C. Binet stage A includes patients with some minimal organomegaly (up to two sites), whilst Rai low risk (formerly stage 0) is restricted to patients without any palpable nodes.

On a historical note, it is worth recalling that after the first International Workshop on CLL (IWCLL) meeting in Paris in 1979 the group proposed to integrate both systems as A (0, I, II), B (I, II) and C (III, IV) [32]. This idea was reiterated in a position paper in 1989 [33]. Despite these publications, the integrated proposal was never implemented in practice, presumably as it was deemed too complex. Clinicians in the USA continue to use the Rai staging and those in Europe the Binet staging. In fact, since both have three stages, consequent differences in patient management are likely to be minimal.

One major issue with both systems is that in patients with early CLL (Rai 0, Binet A) it is difficult to predict the subsequent evolution. In this context, it is interesting to quote from the IWCLL position paper of 1989 [33]: "*Each system has advantages and disadvantages. The Rai system has a precedent, is easily understood, and identifies a subset of patients (stage 0) unlikely, in most instances, to require therapy or to die from chronic lymphocytic leukemia. The disadvantages of the system are the number of disease*

Table 3.3 Clinical staging systems

		Haemoglobin g/L		Platelets × 10^9/L
Rai staging [13, 30][a]	*Lymph nodes/spleen/liver*			
Low risk (formerly stage 0)	Not palpable	≥110		≥100
Intermediate risk (formerly stages I and II)	Palpable nodes and/or spleen and/or liver enlargement	≥110		≥100
High risk (formerly stages III and IV)	Palpable or not	<110	and/or	<100
Binet staging [14]	*Areas of involvement*[b]			
A	0, 1 or 2	≥100		≥100
B	3 or more	≥100		≥100
C	Palpable or not	<100	and/or	<100

[a]Update

[b]Five areas are considered: head and neck, including Waldeyer's ring; axillae; groin, including superficial femorals; palpable spleen; liver (clinically enlarged)

stages (five) and its failure to distinguish different prognostic groups in some studies. The Binet system is a better discriminator of prognosis and is more easily applied to clinical trials and therapeutic strategies (three stages). The Binet system does not identify patients who would be assigned to the Rai stage 0 subset. Also, both systems fail to consider adequately the dynamic nature of the disease. It is reasonable to use either staging system;"…. "*Some variables not included in either system such as the lymphocyte count or its doubling time, bone marrow histologic patterns, and other variables may provide useful supplementary prognostic criteria.*"

There was an earlier study by the French group which attempted to refine the prognostic value of Binet stage A. Cases with a haemoglobin level ≥120 g/L and a lymphocyte count <30 × 10^9/L were distinguished from those with a haemoglobin level <120 g/L and/or a lymphocyte count ≥30 × 10^9/L. There was a significant difference in prognosis between these two groups in the 309 patients studied, with the first group having better OS at 5 years and slower disease progression [34]. This finding was confirmed with data from the MRC CLL1 trial in 606 pts [35].

In the UK clinical trials, the Binet system was used for identifying a subset of patients, "stage A-progressive", as a group requiring treatment. We defined this group by the presence of at least one of the following: a persistent rise in lymphocyte count with doubling time <12 months; a downward trend in haemoglobin or platelets, or both; more than 50% increase in the size of liver, spleen or lymph nodes, or appearance of these signs if not previously present; constitutional symptoms attributable to the disease [36]. In the LRF CLL4 trial, the features which most commonly defined stage A-progressive were a short lymphocyte doubling time and an increase in organomegaly. The clinical justification for defining stage A-progressive as a separate group, meeting the criteria for trial entry, is illustrated in Fig. 3.4, in which the combined data from two large clinical trials (MRC CLL3 and LRF CLL4) show no difference in OS between Stage A-progressive and Stage B.

One important point when staging a patient as Binet C (or Rai high risk, formerly III or IV) is to establish the cause of the cytopenia: either heavy/diffuse BM involvement or an autoimmune process. The former reflects disease burden, whilst the latter may reflect AHA, ITP or the rare PRCA, due to autoantibodies. Data from a large retrospective study at the Mayo Clinic, comprising 1750 CLL patients of whom 24% had cytopenia, showed that 75% of cytopenias were due to BM failure, and 25% due to one of the autoimmune

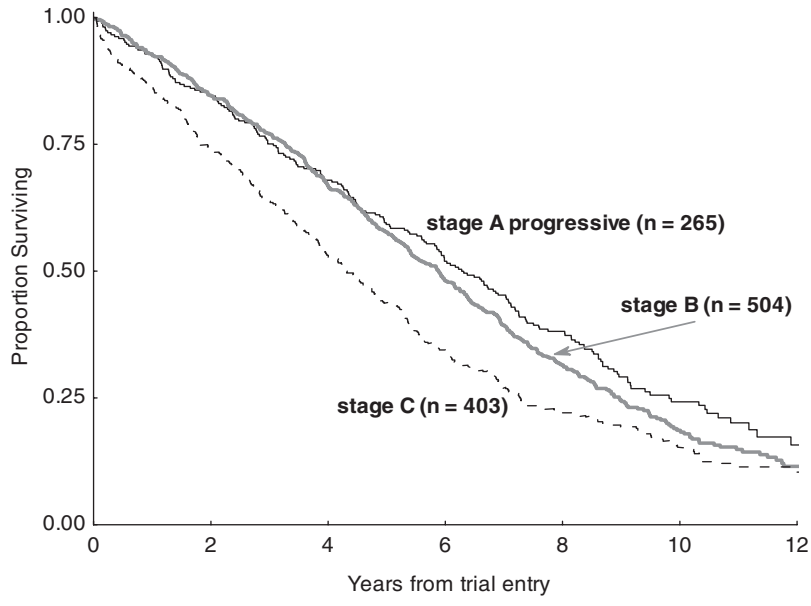

Fig. 3.4 UK trials MRC CLL3 and LRF CLL4: overall survival from trial entry by Binet stage. Log rank test: B vs. A progressive, not significant; B vs. C, $p = 0.0005$; C vs. A progressive, $p < 0.0001$

diseases. The main finding was that those due to autoimmune disease had a significantly better OS than those due to BM burden by CLL [37].

The CLL-IPI prognostic scoring system includes stage as one of the five prognostic criteria by integrating Rai I-IV and Binet B/C, with a score of 1. In contrast, high serum B2M and unmutated *IGHV* genes score 2 each and *TP53* mutation/deletion scores 4 [7] (see Chap. 4).

When dealing with elderly patients it is always necessary to exclude other causes of anaemia, such as iron or folate deficiency, another malignancy, or renal failure. Some of these may be easily corrected with the appropriate haematinics before deciding that the patient needs specific treatment for CLL.

3.6 The Patient's Perspective

Patients may present with quality of life impairment, due in large part to fatigue, with or without anaemia. This may impact adversely their ability to undertake their normal roles and activities [38]. In addition, susceptibility to infections may constrain social and family life. In this context, stage A patients and their families often suffer emotional stress, finding it difficult to accept why, after a diagnosis as serious as leukaemia, treatment is being withheld [39]. They may find it helpful to receive a leaflet explaining why their prognosis is better without treatment and also guidance about what steps they themselves can take to improve their general health and wellbeing. Patients requiring treatment can be reassured that achieving a sustained remission is likely to allow them to return to normal living [38].

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Part IV

Prognostic Markers

Prognostic Markers

4

Anna Schuh

4.1 Introduction

CLL is clinically heterogeneous. One-third of patients never require any therapy and those who do show a highly variable response to chemo-immunotherapy. Long-term follow-up of the German CLL8 Study [1] and the MD Anderson [2] cohorts has demonstrated that about 15% of patients are likely to be cured with chemo-immunotherapy, whereas another 25% of patients relapse within 2 years from finishing therapy. A myriad of biological prognostic markers for CLL have been identified in the past 20 years [3]. However, apart from single nucleotide variants, small insertion/deletions, and deletions affecting the *TP53* locus, none are currently used to direct therapy in routine clinical practice.

It is important to distinguish between prognostic and predictive biomarkers. Prognostic biomarkers clearly define specific clinically relevant subtypes of CLL characterised by distinct clinical outcome. Prognostic markers help to predict the natural history of the disease, are largely independent of treatment interventions, and are identified through the study of longitudinal cohorts of patients with the same disease over a long period of time. With respect to CLL, prognostic markers may be useful to decide on the intensity of follow-up and to predict time to first treatment (TTFT), to estimate the overall survival (OS) from diagnosis, and to evaluate the risk of future high-grade transformation to Richter's syndrome (RS). Later in the disease course, a prognostic marker might assist in deciding whether or not a patient should be referred for peripheral blood stem cell transplantation. A prognostic marker should not only show a statistically significant association with TTFT or OS, but it should also reflect particular biological characteristics and molecular mechanisms that allow a molecular-based sub-classification of the disease.

On the other hand, predictive markers inform treatment choices and are used to direct therapy. They are therefore generally specific to certain classes of drug treatment and used to predict overall response and progression free survival (PFS). The identification of predictive markers requires the study of large cohorts of uniformly treated patients with deep clinical outcome data and/or surrogate markers of clinical outcome, for example measurements of minimal residual disease. In companion diagnostics, predictive markers closely reflect the principle modes of action of the therapies they are linked to and some also have prognostic relevance.

Importantly, both prognostic and predictive markers have to have high positive and negative

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predictive values to be useful in clinical practice and decision-making in individual patients.

4.2 Clinical Prognostic Markers

4.2.1 Clinical Staging Systems

The two major clinical staging systems were described by Rai [4] and Binet [5, 6] more than 40 years ago and are still used in clinical practice today. They were developed before the emergence of therapies that have the potential to change the natural history of CLL and predict overall survival of patients on the basis of a simple physical examination and the full blood count (FBC) results. According to these clinical staging systems, patients with Binet Stage A have the same OS as aged matched controls. Patients with Binet Stage B and C have an OS of 7 and 2 years, respectively. Similarly, patients with Rai Stage 0, 1, 2, 3, and 4 have an OS of 150, 101, 71, 19, and 19 months, respectively. For details of clinical staging see Chap. 3.

4.2.2 Clinical Prognostic Scores

Since then, a number of groups have attempted to improve clinical prognostication in the era of chemo-immunotherapy [7–9] (reviewed [10] and Table 4.1). Most recently, an international effort used individual patient data from eight. Phase 3 trials from France, Germany, Poland, the UK, and the USA to randomly assign training and internal-validation cohorts to establish a comprehensive easy-to-use prognostic score [13]. Two additional datasets from the Mayo Clinic (Rochester, MN, USA; MAYO cohort) and the SCALE Scandinavian population-based case-control study (SCAN cohort) were used as the external-validation datasets. A total of 3472 treatment-naive patients were included in the full analysis dataset. The median age of patients in the full analysis dataset was 61 years (range 27–86). Five independent prognostic factors were identified in the training dataset: *TP53* status (no abnormalities vs. del(17p) and/or *TP53* mutation), *IGHV* mutational status (mutated vs. unmutated), serum β_2-microglobulin concentra-

Table 4.1 Clinical prognostic scores

	Wierda et al. 2007 [8]	Wierda et al. 2011 [9]	Haferlach et al. 2010 [11]	Rossi et al. 2013 [12]	Pflug et al. 2014 [7]	CLL-IPI Working Group 2016 [13]
N patients	1674	930	399	637	1948	3472
Application	All stages, previously untreated	Early-stage only	Early-stage only	All stages, previously untreated	All stages, previously untreated	All stages, previously untreated
Clinical implications	OS and TTT	TTT	OS and TTT	OS	OS	OS
Factors included	Age, beta2-M, ALC, Hb, gender, Rai stage, involved LNA	Unmutated IGHV, diameter palpable LN, del(11q) or del(17p), involved LNA, LDH	Age, WBC, del(17p), unmutated IGHV, IGHV locus translocation, N cytogenetic aberrations	*TP53* del/mut, *BIRC3* del/mut, NOTCH1 mut, SF3B1 mut, del(11q), trisomy 12 normal genetics, del(13q)	Age, gender, ECOG, thymidine kinase, beta2-M, unmutated IGHV, del(17p), del(11q)	*TP53* del/mut, unmutated IGHV, beta2-M, clinical stage, age
Validation	Concordance Index	Internal	None	Internal	Internal and External	Internal and External
Definition of risk groups	Low Intermediate High	Prognostic Nomogram	Favourable Intermediate Unfavourable	Very low Low Intermediate High	Low Intermediate High Very high	Low Intermediate High Very high

OS overall survival, *TTFT* time to first treatment, *beta2-M* beta2-microglobulin, *ALC* absolute lymphocyte count, *LN* lymphnode, *IGHV* immunoglobulin heavy chain variable region genes

tion (≤3.5 mg/L vs. >3.5 mg/L), clinical stage (Binet A or Rai 0 vs. Binet B–C or Rai I–IV), and age (≤65 years vs. >65 years) (Table 4.2). Using a weighted grading of the independent factors, a prognostic index was derived that identified four risk groups within the training dataset with significantly different overall survival at 5 years: those with low (93.2% [95% CI 90.5–96.0]), intermediate (79.3% [75.5–83.2]), high (63.3% [57.9–68.8]), and very high risk (23.3% [12.5–34.1]) scores (Table 4.3). These risk groups were confirmed in the internal-validation and external-validation datasets. Subsequent studies in additional independent datasets have clearly established the CLL-IPI as an extremely valuable tool for prognostication of OS in clinical practice.

Moreover, the value of the CLL-IPI beyond chemo-immunotherapy in the era of novel small molecule inhibitors has been demonstrated by a number of recent publications, and it is likely that it will retain its prognostic importance [14] in this context.

However, the potential role for novel genomic markers apart from *NOTCH1* and *SF3B1* in prognostication remains to be proven as data were available only for a subgroup of patients in the CLL-IPI study. Laboratory methods of varying sensitivity were not standardised across the different cohorts and neither was the number and type of biomarkers investigated. The CLL-IPI is therefore primarily a clinical prognostic score.

Table 4.2 The CLL international prognostic index: definition of risk scores

Risk feature	Score
TP53 status deleted/mutated	4
IgHV status unmutated	2
Beta 2 microglobuline >3.5 mg/l	2
Clinical Stage Rai 1-IV or Binet B-C	1
Age >65	1

4.3 Biological Prognostic Markers

4.3.1 The Immunoglobulin Locus

The most important independent biological prognostic factor in CLL is the mutation status of the immunoglobulin locus (*IgHV*) [15]. The *IgHV* locus is re-arranged in early B-cell development in the bone marrow and every normal B-cell carries its own specific re-arrangement. Upon activation by antigen, B-cells enter the germinal centres of the secondary lymphoid organs where the intracellular enzyme activation-induced cytidine deaminase (AID) introduces point mutations at the immunoglobulin-variable gene loci. It is generally accepted that upon malignant transformation of CLL and other mature B-cell malignancies, a B-cell carrying a unique *IgHV* re-arrangement with varying degree of somatic hypermutation expands and constitutes the clonal leukaemia population. This expansion is thought to be antigen-driven via the B-cell receptor (BCR) machinery. Seminal work by Hamblin et al. showed that CLL patients with hypermutated *IGHV* defined by less than 98% homology to the germline were predicted to have significantly longer OS compared to patients with unmutated *IgHV* genes, defined by *IgHV* sequence homology of equal to or greater than 98% compared to the germline. Patients with hypermutation but carrying the *IgHV* 3–21 re-arrangement have the same poor prognosis as those with unmutated *IGHV* genes [16, 17].

These differences in clinical prognosis are reflected by differences in epigenetic and transcriptomic signatures of *IgHV* unmutated and hypermutated CLL illustrating the fact that these subtypes of CLL can be regarded as two separate disease entities [18–20].

Table 4.3 The CLL international prognostic index: definition of risk groups with associated overall survival

CLLIPI	Risk score	Incidence (%)	Overall survival at 5 years (%)	Median OS (months)
Low	0–1	28–32	93.2	NR
Intermediate	2–3	34–39	79.3	105
High	4–6	25–28	63.3	75
Very high	>6	5–9	23.3	29

Recently, several different groups have shown independently that up to 40% of patients with CLL carry not just one *IgHV* clone but a number of different subclones from different families. These are not oligoclonal expansions of non-malignant B-cells, but they form part of the malignant CD5 positive clonal population [21, 22]. This provides evidence that the early CLL initiating events must pre-date the immunoglobulin re-arrangement in B-cell development. Moreover, patients with multiple unmutated subclones fare worse than those with a single unmutated clone. Conversely, patients with multiple hypermutated subclones fare better and those with a mixture of unmutated and hypermutated subclones have an intermediate TTFT and OS. *IgHV* analysis by next-generation sequencing therefore further refines the sub-classification of CLL according to the *IgHV* mutation status [23].

Importantly, long-term follow-up data of independent cohorts of patients treated with FCR chemo-immunotherapy shows that about 50% of patients with hypermutated *IgHV* status (i.e. approximately 15% of patients requiring frontline therapy in these series) may be functionally cured. In the German CLL8 trial it could be demonstrated that this cohort was enriched for patients with isolated deletion of chromosome 13q14.1 [1].

This observation has important consequences as it means that, for the first time, we are able to identify a group of patients with CLL who might be cured with conventional chemo-immunotherapy.

4.3.2 β2-Microglobulin

β2-microglobulin (β2M) is a component of the major histocompatibility complex (MHC) class I protein, present on the surface of all nucleated cells. It is also found as a free molecule in the surrounding serum. Levels of serum β2M were found to be elevated in CLL patients compared to age-matched controls (median 5 mg/L and 2 mg/L, respectively). Patients with a β2M level > 4mg/L had a shorter OS compared to patients with <4 mg/L (12 months and 43 months, respectively) [24]. Increased β2M levels also indicate shorter TTFT, compounded by a lower probability of achieving complete remission with combination therapies such as fludarabine–cyclophosphamide–rituximab (FCR) [25–27]. More recent studies of larger cohorts have confirmed β2M serum levels as an independent marker of shorter PFS, OS, and poor treatment response and have correlated β2M serum levels with other markers of poor prognosis, including unmutated *IGHV* genes and high CD38 levels [28–30].

4.3.3 Genetic Prognostic Markers

CLL, like all other haematological malignancies and cancers, is an acquired genetic disease. This means that it is ultimately caused by changes called mutations in the genetic material (DNA) of the leukaemia-initiating cell. These lead to abnormal cell differentiation, defects in cellular functions and signalling, increase in proliferation, and decrease in programmed cell death. These mutations can be of two types: First, they may be structural variations such as copy number aberrations (CNAs) defined by loss or gain of part of a chromosome or of entire chromosomes, or translocations. Second, they may be single nucleotide variants (SNVs) that are characterised by single base pair substitutions or insertion/deletions (indels), i.e. losses or gains of up to 20 base pairs. Fluorescent in situ hybridisation (FISH) is routinely used in diagnostics to reveal CNAs and translocations, whereas SNVs and indels can be identified using sequencing.

4.3.4 Copy Number Aberrations

Recurrent CNAs are common in CLL and have been shown to have a significant impact on the prognosis of the disease [31].

4.3.5 Deletions of chromosome 13q14.1

Deletions of the q-arm of chromosome 13 are the most frequent chromosomal aberration in CLL, occurring in 30–55% of cases [31–34].

Del(13q14.1) offers the best prognostic outlook with longer PFS and OS than in patients with normal karyotype [31, 32]. Although the size of the deletion differs from patient to patient, the minimal deleted region (MDR) contains the deleted in lymphocytic leukaemia 2 (*DLEU2*) locus, which encodes the long non-coding RNA (lncRNA) *DLEU2*, the microRNA cluster *MIR15A–MIR16-1*, the *DLEU1* lncRNA gene, and, in some instances, the *DLEU7* gene, which encodes a putative negative regulator of the nuclear factor-κB (NF-κB) transcriptional complex [35–37].

Although del(13q14) is generally associated with a favourable prognosis, the 20% of CLL patients with deletions extending to the retinoblastoma 1 (*RB1*) tumour suppressor gene have a less favourable outcome [36, 37].

The main mechanism by which miR-15a–miR16-1 exerts their tumour suppressor role in B-cells was demonstrated in vivo, where their reintroduction into a human CLL cell line homozygous for del(13q14) led to cell cycle arrest, consistent with their inhibitory action on the expression of multiple genes involved in the G0–G1 transition [38]. It has also been suggested that miR-15a–miR16-1 has a role in the regulation of BCL-2 expression, consistent with the high expression of BCL2 in CLL and the consequent resistance to apoptosis [39].

4.3.6 Deletions of 11q22.3 and ATM Inactivation

Full or partial deletions of the q-arm of chromosome 11 (del11q) are the second most frequent chromosomal aberration found in CLL, occurring in 10–20% of patients [31, 33, 34, 40, 41]. In the pre-rituximab era, the presence of del(11q) was associated with rapid disease progression [31, 42] and significantly lower OS rates than that of patients with normal karyotype CLL [31, 41]. With the addition of rituximab to the chemotherapy backbone, del(11q) CLL without additional poor prognostic markers has become standard risk with up to 40% of patients achieving complete remission [43–45]. Interestingly, recent data have shown that the percentage of del(11q) positive CLL cells present in a patient can affect the disease course. Patients with reduced numbers (<40%) of del(11q) containing cells show both longer TTFT (44 months vs. 19 months) and OS (157 months vs. 90 months) compared to those with higher (≥40%) levels of del(11q) cells [40].

The chromosomal region most commonly deleted as a result of del(11q) known as the minimally deleted region (MDR) is a small section located at the q22.3 band of chromosome 11. Specifically, this is the location of the tumour suppressor gene ataxia telangiectasia mutated (*ATM*), which encodes a protein that is crucial for the cellular response to DNA damage [46, 47]. More than one–third of CLL cases with del(11q22.3) also carry mutations on the remaining ATM allele, indicating that this gene is a major target of these deletions [48, 49]. In a smaller proportion of cases, a single copy of the *ATM* gene is affected by mutations in the absence of genomic deletion, suggesting the possibility of a haploinsufficient tumour suppressor role of ATM [50].

Similar to cases with TP53 disruption, ATM-disrupted CLL is associated with genomic instability, the acquisition of additional genetic lesions, and chemo-resistance. Small molecule inhibitors of ATR, PARP, CHK1/2, or dual TORK/DNA-PK targeting mutations in *ATM* and other DNA damage response genes by producing synthetic lethal states have shown promising preclinical activity and are currently undergoing evaluation in early phase clinical trials [47].

4.3.7 Trisomy 12

Gains of chromosome 12 (trisomy 12) are found in 10–17% of CLL cases [31, 33, 34] and confer an intermediate risk, with PFS similar to patients with normal karyotype by interphase FISH [31, 32]. Mutations in the *NOTCH1* gene have been associated with trisomy 12 in CLL [51, 52] leading to a significantly shorter OS compared to trisomy 12 cases carrying wild-type *NOTCH* [53].

The presence of trisomy 12 in CLL has been linked to disease progression, specifically the development of Richter's syndrome (RS) [54].

Despite its recurrence and prognostic importance, the mechanisms by which trisomy 12 contributes to CLL pathogenesis remain unknown.

4.3.8 Del(17p) and TP53 Inactivation

Prior to initiation of frontline therapy, deletions of the p-arm of chromosome 17 and *TP53* mutations are found in 4% [31, 32] and 8–12% [55] of CLL cases, respectively. The majority of cases with a deletion of one allele carry a single nucleotide variant mutation or small insertion/deletion of *TP53* on the other allele. In the relapse setting, the incidence of *TP53* abnormalities including del17p and/or mono-allelic or bi-allelic *TP53* mutation has been reported to be as high as 25% [56, 57]. Both deletions and *TP53* single nucleotide variants (SNVs) and indels are associated with poor responses to DNA-damaging chemotherapeutic regimens, consistent with their dominant-negative function [45, 58, 59], and confer a poor prognosis, with rapidly progressing disease and reduced OS and TTFT.

Patients with del(17p) demonstrate low response rates to many chemotherapy regimens [29, 43, 59–62], with combination therapies offering no significant improvement. The prognostic significance of isolated del(17p) without *TP53* mutation remains uncertain.

More recently, sub-clonal *TP53* mutations were also shown to be associated with short OS and early relapse following chemo-immunotherapy [57, 63–65]. They should therefore be tested for with sensitive and standardised methods in routine clinical settings, especially since novel and effective agents are now available in the clinic that bypass TP53 and induce cell death via TP53 independent pathways.

4.3.9 Complex Karyotype

The extreme form of copy number aberration is called a complex karyotype meaning a high number of losses or gains affecting several chromosomes within the same cell.

Complex karyotype is a feature of genomic instability and in CLL it is strongly associated with mutations of *TP53* or in other genes involved in the DNA damage response such as *ATM*.

The precise definition and incidence of complex karyotype heavily depends on the method used to reveal losses and gains of chromosomes in CLL cells. Stimulation of leukaemia cells with mitogenic agents followed by karyotyping of at least 20 metaphases was the first method used. Despite optimisation of culture conditions and reagents, it has a number of significant technical challenges and is therefore not part of routine diagnostics. Alternative methods such as genome-wide array technology (comparative genomic hybridisation and single nucleotide polymorphism arrays) have been evaluated as research tools and reliably detect CNAs in all CLL cells including those that do not divide without the need for stimulation. Whatever technology is used, there is currently a lack of standardisation. This is important as sensitivities of the different technologies directly impact on the number and size of chromosomal losses and gains detected.

4.3.9.1 Recurrent Acquired Single Nucleotide Variants (SNV) in CLL

Explorative genome-wide [66–69] and exome-wide [65, 70–72] massive parallel sequencing efforts have identified a number of additional recurrent acquired mutations in the coding regions of genes in CLL cells. The vast majority of these occur at low frequency and their potential prognostic significance remains unknown. Here, we therefore focus on genes that are recurrently mutated in over 10% of CLL patients before initiation of frontline therapy.

NOTCH1

The NOTCH family is a highly conserved group of genes that are critical in regulating haematopoiesis and helping to mediate cell fate and diversity [73]. The NOTCH signalling pathway is involved in a number of crucial cell functions, including proliferation, cell differentiation and apoptosis. NOTCH1 is a transmembrane protein that cleaves its intracellular domain ($NOTCH1^{IC}$)

upon activation by an extracellular ligand. NOTCH1^{IC} translocates to the nucleus and forms a complex with CBF1, MAML, and p300 to become a transcriptional activator for NOTCH target genes. NOTCH1 is both upregulated and constitutively activated in CLL cells compared to normal controls and is associated with apoptosis resistance [66, 74–77]. A 2bp deletion in the NOTCH1 PEST domain (del7544_45) is found in 4–12% of CLL cases and results in early termination of the PEST domain required for proteosomal degradation by the ubiquitin ligase F-box and WD repeat containing protein 7 (FBXW7) that is also targeted by recurrent inactivating mutations in CLL. As a result, NOTCH1^{IC} accumulates in the nucleus leading to constitutive activation of NOTCH1 target genes.

More recently, recurrent mutations in the 3′ UTR of *NOTCH1* that lead to aberrant splicing events disrupting the PEST domain were found in an additional ~3% of patients with CLL [68].

Patients carrying mutations in the PEST domain of NOTCH1 did not benefit from the addition of anti-CD20 therapy to the chemotherapy backbone [78]. A likely explanation might be that patients with CLL and *NOTCH1* mutations have significantly lower CD20 expression on CLL cells compared to CLL cells without *NOTCH1* mutation [79].

NOTCH1 mutations have also been associated with an increased risk of transformation of CLL into diffuse large B-cell lymphoma (DLBCL) (see Chap. 10) [80].

SF3B1

Splicing factor 3B subunit 1 (SF3B1) forms part of the U2 small nuclear ribonucleoprotein complex that plays a role in mRNA splicing. Mutations in *SF3B1* are present in 10–20% of cases [70, 71, 78, 81–83] and are typically missense variants clustered in the highly conserved HEAT domain of SF3B1, with 42–50% of mutations affecting the lysine residue at position 700. This recurrently affected region is predicted to form the inner surface of the SF3B1 protein and therefore may represent disruption of a binding site, leading to the altered splicing function seen in these cases [67]. Mutations in *SF3B1* tend to be sub-clonal and expand over time contributing to disease progression, particularly following chemotherapy.

4.3.9.2 Hierarchical Model Integrating Prognostic Information from Genomic Studies

A pivotal training-validation study carried out on >1000 newly diagnosed and previously untreated patients with CLL from the North Italian Registry proposed a combined hierarchical model of prognostically relevant SNVs revealed by sequencing and CNVs detected by traditional fluorescence in situ hybridisation (FISH) that lead to the following classification schema: high-risk CLL (i.e. *TP53* and/or *BIRC3* disrupted); intermediate-risk CLL (i.e. *NOTCH1* and/or *SF3B1* mutated and/or del(11q)); low-risk CLL (trisomy 12 or patients with normal karyotype); and very low-risk CLL (only del(13q14)) [12]. Applying the model to this specific patient cohort, ~20% of patients belonging to the low-risk categories on the basis of the FISH-based hierarchical model were reclassified into higher-risk categories owing to the presence of *NOTCH1, SF3B1,* or *TP53* mutations or *BIRC3* disruption, thus significantly improving the accuracy of prediction of clinical evolution. Subsequently, a number of different studies using a number of independent non-trial patient cohorts attempted to validate this initial hierarchical model [81, 84, 85], but results remained in part inconclusive due to the different types of patient populations included, various laboratory techniques of variable sensitivity used, the number of genes interrogated, and varying incidence of mutations in the different study groups. However, in multivariate analysis, all three studies were able to confirm the poor prognostic value of *SF3B1* and *IgHV* mutation status with regards to TTFT. Two [81, 85] of the three studies also described unmutated *IgHV* genes and the presence of *SF3B1* or *TP53* mutations as independent predictors of poor OS.

All the studies conclude that while the integration of genomic data into prognostic model systems remains a promising goal, more data from well-controlled cohorts and uniformly treated patients within clinical trials using standardised

laboratory methods are required before hierarchies of biomarkers can be introduced into routine clinical practice to aid clinical decision-making.

4.3.9.3 Predicting the Risk of High-Grade Transformation

There are several biological risk factors associated with progression of CLL to RS. Genetic defects, such as mutations and deletions of the tumour suppressor gene *TP53, p16INK4A,* or p21 and loss of p27 expression, BCL2 overexpression, overexpression and genotype of CD38, ZAP70, unmutated *IgHV* genes, and *IgHV* gene usage, have been implicated in progression of CLL to RS across several studies [86, 87]. Of these, genotype and expression for CD38, the absence of del(13q14), and the *IgHV4-39* rearrangement were independent risk factors.

In particular, *TP53* abnormalities are common in RS. Rossi et al. found that 47.1% of RS patients displayed TP53 deletion, mutation, or both. A similar proportion of patients with *TP53* disruption was confirmed prospectively in the CHOP-OR trial [88]. *TP53* abnormalities have been implicated in the progression of CLL to clonally related RS and are associated with poor prognosis in all RS subgroups [54]. Subsequently, a prognostic scoring system was developed that includes (1) *TP53* disruption, (2) Eastern Cooperative Oncology Group (ECOG) performance status, and (3) response to induction therapy [87].

In addition, it was shown that alongside *TP53* disruption, *CDKN2A* loss is a key candidate driver of transformation and is present in approximately 50% of RS cases [54, 87]. Whereas *TP53* disruption was often present *prior* to transformation, *CDKN2A* loss occurred typically *at* transformation. There was a clear association with c-MYC overexpression and *TP53* disruption in some cases providing a classical oncogenic combination.

Other CNAs have been described at a significantly higher frequency in RS compared to CLL in particular 15q (*MGA*) losses and 2p gains (*MYCN* and *REL*). Moreover, patients with a *NOTCH1* mutation have been shown to be significantly more likely to transform to RS (45% with *NOTCH1* mutations versus 4% without). The group of RS with trisomy 12 (associated with *NOTCH1* mutations) is mutually exclusive from those harbouring *TP53/CDKN2A/c-MYC* genetic abnormalities [54].

4.4 Prognostic Markers in the Era of Novel Therapies

The emergence of small molecules targeting either B-cell receptor signalling via Bruton kinase (BTK) [14, 89, 90] or phosphoinositide 3-kinase (PI3K) [91] inhibition or the anti-apoptotic pathway via inhibition of BCL-2 [92] has revolutionised the treatment of chemo-immunotherapy-resistant CLL including for patients with *TP53* disruption. However, the median PFS for these high-risk patients treated with single agent ibrutinib is 28 months, compared to 38 months for patients with del(11q) and not reached at 3 years for patients with neither of these abnormalities. The vast majority of patients with either del(17p) or del(11q) also have a complex karyotype. Consistent with these results, patients with complex karyotype were shown to have a short time to relapse on ibrutinib [93]. In this single centre study from the MD Anderson, 17 out of 21 patients with a complex karyotype also had concurrent del(17p) making an assessment of independent prognostic value impossible. It also remains to be seen whether the relatively worse outcome of patients with del(11q) treated with single agent ibrutinib can be improved by the addition of rituximab.

Follow-up of the largest cohort of Ibrutinib-treated patients so far ($n = 308$) [94] identified 31 patients who had discontinued therapy because of disease progression (PD), and 45 had discontinued for other reasons. PD included RS ($n = 18$) or progressive CLL ($n = 13$). Significantly associated predictors of PD were the number of prior therapies (HR, 1.12; $P = 0.03$), *BCL6* abnormalities (HR, 3.77; $P < 0.001$), *MYC* abnormalities (HR, 2.59; $P = 0.01$), presence of del(17p) (HR, 2.28; $P = 0.03$), and complex karyotype (HR, 5.17; $P = 0.003$). In multivariable analysis, presence of *BCL6* abnormalities (HR, 2.70; 95% CI, 1.25–5.85 [$P = 0.01$]) and complex karyotype (HR, 4.47; 95%CI, 1.50–13.34 [$P = 0.007$]) remained independent risk factors.

Interestingly, a complex karyotype did not predict outcome following treatment with idelalisib.

With respect to CLL progression, drug-resistance mutations in the BCR pathway genes including the BTK binding site of ibrutinib or gain-of-function mutation in *PLCG* were the major cause of resistance development. Using deep sequencing, these mutations were not detected before the start of ibrutinib therapy indicating that they occurred under the selective pressure of the drug. Similarly, in patients who develop resistance to the BCL-2 inhibitor venetoclax, mutations of bcl2 family proteins have been observed [95]. It remains to be seen whether molecular monitoring and pro-active treatment of molecular relapse should have a place in CLL therapy.

4.5 Outlook

Using massively parallel sequencing, recent international efforts have revealed the complex genomic landscape of cancers including haematological malignancies. Specifically for CLL, we have compiled a comprehensive catalogue of somatically acquired mutations in the protein coding regions that represent less than 1% of the genome [70, 72, 96]. Only *TP53, ATM, SF3B1,* and *NOTCH1* carry coding mutations in over 10% of cases. The other genes are infrequently affected by coding mutations possibly reflecting the heterogeneous nature of CLL, and large cohorts of patients will be required to establish the clinical significance of these mutations. Only one study so far has comprehensively investigated uniformly treated patients with robust clinical outcome data as part of a clinical trial [65]. This study revealed coding mutations in *RPS15* in addition to mutations in *TP53* as predictors for short PFS following chemo-immunotherapy. A small number of patients have undergone whole genome sequencing [66, 68, 69, 97]. One of these studies revealed for the first time the function and biological relevance of non-coding mutations in *NOTCH1* and *PAX5* [68]. For non-coding *NOTCH1* mutations, a link to poor clinical outcome could be established [98]. In addition, whole genome sequencing has the potential to reveal global mutation signatures [97, 99], subclonal architecture [69, 70], and absolute mutational load. These have already been linked to clinical outcome. Although the cost of sequencing, bio-informatics analysis, and clinical interpretation of whole genome sequencing remains prohibitive, it is likely that over the next few years the systematic interrogation of the CLL genome in the context of clinical trials will refine our current approaches to response prediction and prognostication.

In summary, prognostic testing in CLL might be valuable in early-stage disease, because of different follow-up strategies [100] and possibly earlier treatment initiation of very high-risk CLL within a clinical trial. The potential consequence of the screening results on follow-up, therapy, and quality of life should be discussed with the patient before prognostic testing. Particularly in older and more comorbid patients, prognostic marker results may not result in any consequences until the patient becomes symptomatic. Hence the benefit of prognostic markers analysis in early-stage CLL of older and comorbid patients should be discussed individually. At the time of treatment initiation, comprehensive risk factor assessment, including genetic markers, is generally recommended for all patients because of therapeutic implications. Complex karyotype and multiple genetic mutations result in poor prognosis, even with novel agents. Therefore, these additional tests should be considered in patients fit enough for allogeneic stem cell transplantation and experimental protocols. Together with the continuous development of novel therapies and treatment approaches, scoring systems and prognostication are expected to undergo dramatic changes during the next years. Regardless of the laboratory methods chosen to interrogate disease biology, a systematic approach from unbiased biomarker discovery to technical and clinical biomarker validation has to become an integral part of conducting clinical trials in the era of precision medicine. Altogether this holds the promise that a more individualised treatment approach on the basis of prognostic profiles will be possible.

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Part V

Treatment

5 Guidelines for Diagnosis, Indications for Treatment, Response Assessment, and Supportive Management of Chronic Lymphocytic Leukemia: *The 2018* Update

Barbara Eichhorst and Michael Hallek

5.1 Introduction

In 2008, the International Workshop on CLL (iwCLL) published consensus guidelines for the design and conduct of clinical trials for patients with chronic lymphocytic leukemia (CLL) based on previously published recommendations by a National Cancer Institute-sponsored Working Group [1–3]. These guidelines provided definitions regarding the assessment of CLL patients that were adopted by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the evaluation of new drugs. Major advances in the biology and treatment of patients with CLL in the last decade prompted the iwCLL to re-evaluate and revise the 2008 criteria.

In the 2018 update of the guidelines, the following major changes or additions were introduced:

- Description of the clinical relevance of recent discoveries on the genomic alterations found in CLL, including mutations of the *TP53* gene.
- Account of the increasingly important prognostic role of the IGHV mutational status.
- Recommendations regarding the current use of clinical staging, novel genetic or biological prognostic markers, and prognostic scores.
- Harmonization of the assessment of splenomegaly, hepatomegaly, and lymphadenopathy with the relevant sections of the updated lymphoma response guidelines.
- Update of the response assessment for novel targeted drugs (kinase inhibitors and Bcl2 inhibitors) that are often evaluated during continuous therapy.
- Account of the increasingly important role of assessing minimal residual disease.
- Recommendations regarding the baseline assessment and prophylaxis of viral diseases before and under therapy of CLL.

Because the full paper is now published [4], this review paper summarizes the major changes and adaptations, as well as the indications for treatment initiation and response evaluation.

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5.2 Clinical Relevance of Genetic or Genomic Alterations Found in CLL, Including Mutations of the *TP53* Gene

Interphase fluorescence in situ hybridization (FISH) can be performed with peripheral blood lymphocytes and identifies cytogenetic lesions in more than 80% of all CLL cases [5]. The most common deletions are in the long arm of chromosome 13 (del(13q)). Additional, frequent chromosomal aberrations comprise trisomy of chromosome 12, deletions in the long arm of chromosomes 11 (del(11q)), and in the short arm of chromosome 17 (del(17p)) (see also Chap. 4) [5].

Appropriate stimulation of CLL cells in vitro has enabled the performance of conventional karyotyping with enhanced reliability [6]. With this methodology additional chromosomal aberrations of potential prognostic significance can be identified [6–8]. Moreover, stimulated metaphase karyotyping has demonstrated that leukemia cells with a complex karyotype (i.e., three or more chromosomal abnormalities) may have adverse prognostic significance [9–12]. However, more data from prospective trials are needed to validate the prognostic and predictive value of stimulated metaphase karyotyping before it can be recommended for routine practice.

So far, other technologies array-based assays or next-generation sequencing has not been able to completely replace FISH or conventional karyotyping.

Certain genetic abnormalities are associated with adverse outcome in response to standard chemo(immuno)therapy. It has been demonstrated that the progression-free survival and overall survival of CLL patients carrying a del(17p) and patients carrying a *TP53* mutation as detected by Sanger sequencing in the absence of del(17p) are similar [13]. Therefore, the assessment of both del(17p) and *TP53* mutation has prognostic and predictive value and should guide therapeutic decisions in routine practice.

Patients who have leukemia cells with del(17p) and/or *TP53* mutation also respond poorly to chemoimmunotherapy but fare significantly better when treated with non-chemotherapeutic agents, such as small molecule inhibitors of BTK, PI3K, or BCL2 (see also Chap. 7). For clinical trials, it is recommended that molecular genetics be performed prior to treating a patient on protocol. As additional genetic abnormalities may be acquired during the course of the disease [14], genetic analyses (in particular for del(17p)/*TP53* mutations) should be repeated prior to any subsequent, second- or third-line of treatment.

Next-generation whole exome or whole genome sequencing has identified additional genomic abnormalities, such as mutations in *NOTCH1* or *SF3B1*, that have pathogenic as well as prognostic significance. However, more data from prospective trials are needed to validate the prognostic and predictive value of these genomic abnormalities before we can advocate using them in routine practice.

5.3 Prognostic Role of the IGHV Mutational Status

The leukemia cells use immunoglobulin variable heavy chain (IGHV) genes that may or may not have undergone somatic mutations [15–17]. The outcome of patients with leukemia cells that use an unmutated IGHV gene (usually defined as 98% or more sequence homology to the nearest germline gene) is inferior to that of patients with leukemia cells that use a mutated IGHV gene (see also Chap. 4) [18, 19]. Moreover, the presence of mutated IGHV genes, in particular when combined with additional prognostic factors such as favorable cytogenetics or attainment of a minimal residual disease negative state after therapy, characterizes a CLL patient subgroup with excellent outcome following chemoimmunotherapy with fludarabine, cyclophosphamide, and rituximab [20–22].

The discovery of almost identical or "stereotyped" B-cell receptor immunoglobulins among unrelated CLL patients suggests that (auto) antigen selection may play a role in disease pathogenesis [23]. Approximately one-third of patients can be grouped into subsets based on shared sequence motifs within the immunoglobulin heavy chain variable region (IGHV) complementarity determining region 3 (CDR3) [23]. It seems that some of these subgroups share a similar prognosis. For example, *IGHV3-21* gene

usage (of stereotype subset 2) may be associated with an unfavorable prognosis independent of the IGHV mutational status [24, 25]. As of today, assessment of IGHV stereotypes is not an element of the routine prognostic work up in CLL.

5.4 Recommendations Regarding the Clinical Use of Clinical Staging, Novel Genetic or Biological Prognostic Markers, and Prognostic Scores

In daily practice, Rai or Binet stages help stratify patients according to the disease risk. However, there are a large number of biomarkers that can provide additional prognostic information [26–28]. The most relevant prognostic parameters are *IGHV* mutational status, serum ß$_2$-microglobulin, and the presence of del(17p) and/or *TP53* mutations. Usually, high-risk CLL is defined, at least in part, by a genetic aberration of the *TP53* gene (i.e., del(17p) or *TP53* mutation) (see also Chaps. 3 and 4).

Following the identification of these new prognostic parameters, several prognostic scores and stratification systems, as the CLL-IPI, have been proposed based on multivariate analyses to extract the most significant independent prognostic information from the plethora of known prognostic markers [29–31]. It should be emphasized that the value of prognostic markers or scores might change with the application of novel therapies.

5.5 Assessment of Lymphadenopathy, Splenomegaly, and Hepatomegaly

The assessment of *lymphadenopathy* should always be performed by physical examination. In clinical trials, a CT scan of the neck, abdomen, pelvis, and thorax is desirable before treatment initiation for response evaluation. To be counted as "normal," lymph nodes should be <1.5 cm in longest diameter. For follow-up further imaging is not required for CLL management until disease progression is apparent by clinical examination or on blood testing (see also Chap. 3). Similarly, *splenomegaly or hepatomegaly* should be evaluated by physical examination. In clinical trials, a CT scan of the abdomen should be performed at response assessment and should show no evidence for lymphadenopathy and splenomegaly. The consensus response cutoff for splenomegaly is 13 cm in cranio-caudal length [32, 33]. Importantly, the persistence of splenomegaly after successful therapies may not correlate with outcome [32]. The quantitative determination of hepatomegaly seems more difficult; changes such as focal or disseminated hepatic nodules support liver involvement.

5.6 Indication for Treatment Initiation

In spite of recent advances, newly diagnosed patients with asymptomatic early stage disease (Rai 0 and Binet A) should be monitored unless they develop symptoms of active and/or progressive disease. Prior studies have shown that early treatment with alkylating agents does not translate into a survival advantage in patients with early stage CLL [34–36].

Treatment should be initiated in patients with advanced stage disease (Rai III and IV or Binet C) due to hematopoietic insufficiency (Table 5.1). Patients with intermediate stage (Rai I and II or Binet B) can be monitored until they have symptoms of progression and/or symptomatic disease. According to the IWCLL guidelines the following conditions define active disease [4]:

- Disease-related symptoms:
  - Unintentional weight loss ≥10% within the previous 6 months.
  - Significant fatigue (i.e., ECOG PS 2 or worse; cannot work or unable to perform usual activities).
  - Fevers ≥100.5 °F or 38.0 °C for 2 or more weeks without evidence of infection.
- Bone marrow failure with worsening of anemia and thrombocytopenia not caused by autoimmune phenomena. Cutoff levels of Hb < 10 g/dl or platelet counts of <100,000/µl are generally regarded as indication for treat-

Table 5.1 Treatment indications

	General practice	Clinical trial
Frontline		
Treat with Rai stage 0 or Binet stage A	NGI[a]	RQ
Treat with Binet stage B or Rai stage I or Rai stage II	Possible[a]	Possible[a]
Treat with Binet stage C or Rai stage III or Rai stage IV[b]	Yes	Yes
Relapse		
Treatment of active/progressive disease	Yes	Yes
Treat without active/progressive disease	No	RQ

NGI not generally indicated, *RQ* early therapy of CLL is generally not recommended outside of clinical trials
[a]Treatment is indicated, if the disease is active as defined above in the text
[b]Anemia and/or thrombocytopenia due to CLL-unrelated causes should be excluded

ment. However, it should be pointed out that in some patients platelet counts of <100,000/µl may remain stable over a long-period of time; this situation does not require therapeutic interventions.

- Autoimmune anemia and/or thrombocytopenia poorly responsive to corticosteroids.
- Massive (≥10 cm) or progressive or symptomatic lymphadenopathy.
- Massive (≥6 cm below left costal margin) or progressive or symptomatic splenomegaly.
- Symptomatic or functional extranodal involvement (e.g., skin, kidney, lung, spine).
- Lymphocyte doubling time of less than 6 months or 50% lymphocyte doubling in less than 2 months (patients with initial <30 G lymphocytes/L may require longer observation period).

These criteria may also lead to treatment initiation in early stage disease. The absolute lymphocyte count itself should not be used as a sole indicator for treatment initiation, because even patients with very high lymphocyte counts rarely develop symptoms.

Disease relapse alone is not an indication for retreatment. Many patients are asymptomatic with relapsed disease and therefore do not require treatment. Subsequent treatment decisions should generally follow the same indications as those used for first line therapy [4].

5.7 Response Assessment Including Response Assessment for Novel Targeted Drugs (Kinase Inhibitors and Bcl2 Inhibitors)

Due to the advent of novel agents for CLL therapy, treatment is often applied in a continued schedule over several months or even years. Moreover, some agents cause different response patterns with a transient lymphocytosis. Therefore, the guidelines needed some modifications.

Assessment of response should always include a careful physical examination and evaluation of the blood and bone marrow. The timing of response assessment for therapies with a defined treatment duration (such as chemoimmunotherapeutic approaches) should be at least 2 months after completion of therapy. To define the response to therapy, two groups of parameters need to be assessed and documented: parameters of group A assess the lymphoid tumor load and constitutional symptoms, while parameters of group B assess the hematopoietic system (Table 5.2) [4].

For continued therapies or treatment strategies that contain a maintenance phase, the assessment of response should be performed at least 2 months after patients achieve their maximum response or at a time point that is predefined in the protocol; in this case, it is not necessary to interrupt therapy for response assessment. Maximum response can be defined as a treatment phase where no additional improvement is seen during at least 2 months of therapy. The definition of response is the same as with defined treatment duration (Table 5.2). In clinical trials, any response (e.g., CR, PR) should be sustained for at least 2 months prior to using this response in the assessment. In addition, where appropriate, a further assessment of response (i.e., marrow assess-

Table 5.2 Response definition during/after treatment for CLL patients

Group	Parameter	CR	PR	PD	SD
A	Lymphadenopathy	None ≥1.5 cm	Decrease ≥50% (from baseline)[a]	Increase ≥50% from baseline or from response	Change of −49% to +49%
	Liver and/or spleen size[b]	Spleen size <13 cm; liver size normal	Decrease ≥50% (from baseline)	Increase ≥50% from baseline or from response	Change of −49% to +49%
	Constitutional symptoms	None	Any	Any	Any
	Circulating lymphocyte count	Normal	Decrease ≥50% from baseline	Increase ≥50% over baseline	Change of −49% to +49%
B	Platelet count	≥100,000/μl	≥100,000/μl or increase ≥50% over baseline	Decrease of ≥50% from baseline secondary to CLL	Change of −49 to +49%
	Hemoglobin	≥11.0 g/dl (untransfused and without erythropoietin)	≥11 g/dl or increase ≥50% over baseline	Decrease of ≥2 g/dl from baseline secondary to CLL	Increase <11.0 g/dl or <50% over baseline, or decrease <2 g/dl
	Marrow	Normocellular, no CLL cells, no B-lymphoid nodules	Presence of CLL cells, or of B-lymphoid nodules, or not done	Increase of CLL cells by ≥50% on successive biopsies	No change in marrow infiltrate

CR complete remission (all of the criteria have to be met), *PR* partial remission (at least 2 parameters of group A and 1 parameter of group B need to improve if previously abnormal. If only one parameter of both groups A and B is abnormal prior to therapy, only 1 needs to improve.), *PD* progressive disease (at least one of the above criteria of group A or group B has to be met), *SD* stable disease (all of the above criteria have to be met. Constitutional symptoms alone do not define PD)

[a]Sum of the products of 6 or less lymph nodes (as evaluated by CT scans and physical exam in clinical trials or by physical exam in general practice)

[b]Spleen size is considered normal if <13 cm. There is no firmly established, international consensus of the size of a normal liver; therefore, liver size should be evaluated by imaging and manual palpation in clinical trials and be recorded according to the definition used in a study protocol

ment) may be performed at least 2 months after the patient has cleared minimal residual disease from the peripheral blood.

It has been noted that certain therapies (e.g., kinase inhibitors) may cause lymphocytosis. In the setting of therapy with such agents, an increase in blood lymphocyte count, by itself, does not uniformly indicate an increased tumor burden, but may reflect re-distribution of leukemia cells from lymphoid tissues to the blood. In such cases, an increase in the number of blood lymphocytes (defined by 50% or more with at least 5000 B-lymphocytes per μL) is not a sign of treatment failure or progressive disease [37].

5.8 Increasingly Important Role of Assessing Minimal Residual Disease

The complete eradication of CLL is certainly a desired endpoint. Use of sensitive multicolor flow cytometry, PCR, or next-generation sequencing can detect minimal residual disease (MRD) in many patients who achieved a complete clinical response. Prospective clinical trials have new provided substantial evidence that therapies that are able to eradicate MRD usually result in an improved clinical outcome [33, 38–42]. The techniques for assessing MRD have undergone a critical evaluation and have become well standardized [43, 44]. Six-color flow cytometry (MRD flow)

(see also Chap. 2), allele-specific oligonucleotide PCR, or high-throughput sequencing using the ClonoSEQ assay is reliably sensitive down to a level of less than one CLL cell in 10,000 leukocytes [44]. Refinement and harmonization of these technologies has established that a typical flow cytometry-based assay comprises a core panel of six markers (i.e., CD19, CD20, CD5, CD43, CD79b, and CD81) [44]. As such, patients will be defined as having undetectable MRD (MRD-neg) remission if they have blood or marrow with less than one CLL cell per 10,000 leukocytes. The blood generally can be used for making this assessment, as the marrow will have detectable CLL when it is also found in the peripheral blood. However, there are therapies that preferentially clear the blood but not the marrow (such as monoclonal antibodies). Therefore, it may be important to confirm that the marrow aspirate also is MRD-neg when the blood is found to be MRD-neg. Clinical trials aimed at maximizing the depth of remissions should include at least one test to assess for MRD, because the lack of leukemia persistence using these sensitive tests has a strong, positive prognostic impact. The report should be clear as to whether blood and/or marrow have been assessed and should report the proportion of MRD-neg patients on an intent-to-treat basis using the total number of patients in that treatment arm as the denominator (not those assessed or those who responded to treatment).

5.9 Recommendations Regarding Prophylaxis of Infections

Infections are a frequent problem during the management of CLL patients [45–47]. Unfortunately, there are no randomized studies showing that vaccination may alter infection rates or outcomes from acquired infections in CLL. It is generally recommended that routine vaccinations should be performed before initiation of treatment if possible. Vaccinations achieve reasonable rates of seroprotection and seroconversion in immunocompromised cancer patients, with minimal side effects [47]. Conjugate vaccines have proved to be highly immunogenic and are to be preferred, where available, in CLL patients [48]. Vaccines against seasonal influenza and against H1N1 can be recommended, given the severity of the H1N1 pandemic and the highly severe flu impact in immunocompromised CLL patients [49]. Live vaccines are contraindicated in CLL patients, since severe or even fatal complications have been reported [45].

During therapy, particular attention should be given to monitoring for symptoms or laboratory evidence of opportunistic infections such as Pneumocystis jirovecii or herpesviridae (herpes simplex virus, varizella zoster virus, cytomegalovirus, or Epstein Barr virus) in patients treated with agents like alemtuzumab and idelalisib (alone or in combination), or with allogeneic stem cell transplantation. Patients receiving anti-CD20 antibodies may experience reactivation of hepatitis B virus (HBV) infections [50]. Therefore, the HBV serological status should be evaluated prior to treatment with such agents; appropriate antiviral prophylaxis should be initiated in patients with a history of HBV infection [50]. In contrast, the infection rate seems low in patients younger than 65 years treated with fludarabine-based first line therapy, where no monitoring or routine anti-infective prophylaxis is required [51]. Progressive multifocal leukoencephalopathy has been reported in a few CLL patients treated with anti-CD20 antibodies; therefore, infections with John Cunningham (JC) virus should be ruled out in situations of unclear neurological symptoms [52–55].

Hypogammaglobulinemia (low serum levels of IgG and IgA with variable IgM) is a well-recognized complication associated with CLL. Regarding the substitution of CLL patients with hypogammaglobulinemia and history of infections, six randomized studies have shown that the prophylactic use of intravenous immunoglobulins (IVIG) decreases the rate of bacterial infections and prolongs the time to first infection, but does not produce differences in survival or other outcome parameters (summarized in [45]). Therefore, the use of IVIG cannot be routinely recommended but should be reserved to individual situations of hypogammaglobulinemia and repeated infections.

5.10 Summary

Treatment of CLL is undergoing rapid changes, leading to an improved outcome. The updated iwCLL guidelines summarize the current state of the art regarding the diagnosis, indications for treatment, response assessment, and supportive management of CLL patients in light of these changes and aim to harmonize the management and the conduct of clinical trials of this disease worldwide.

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6 Initial Therapy of Chronic Lymphocytic Leukemia

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6.1 Introduction

For patients with chronic lymphocytic leukemia (CLL) treatment was purely palliative from the time when the alkylating agent chlorambucil was introduced in 1956 [1]. In the 1980s combination therapies with cyclophosphamide, doxorubicine, vincristine, and prednisolone (CHOP) or similar regimens, e.g., COP, were compared to chlorambucil within randomized studies, but they were not able to show a clear benefit due to a higher toxicity rate [2–4]. With the introduction of the purine analogues (fludarabine, cladribine, and pentostatin) significantly better response rates as well as longer progression-free survival (PFS) were seen in comparison to CHOP or chlorambucil [5–8]. The combination of purine analogues with cyclophosphamide yielded significantly higher rates of complete remission and, in addition, prolongation of relapse-free time [9–12]. With the addition of rituximab to these regimens, or to single chemotherapeutic agents, CD20 antibody based chemoimmunotherapy became the standard treatment in previously untreated CLL without TP53 mutation or deletion. The new targeted treatment agents, such as Btk-inhibitors or bcl2-inhibitors, approved mainly for the relapsed and refractory setting (see also Chap. 8) and currently being investigated in several studies for upfront treatment, will change frontline therapy of CLL in the near future.

This chapter summarizes current treatment options including chemoimmunotherapy as well as chemotherapy-free regimens.

6.2 Indication for Treatment Initiation

In general, newly diagnosed patients with asymptomatic early stage disease (Rai 0, Binet A) should be monitored unless they develop symptoms of active and/or progressive disease. Prior studies have shown that early treatment with alkylating agents does not translate into a survival advantage in patients with early stage CLL [13–15].

Treatment should be initiated in patients with advanced stage disease (Rai III and IV or Binet C) due to hematopoietic insufficiency. Patients with intermediate stage (Rai I and II or Binet B) can be monitored until they have symptoms of progression and/or symptomatic disease.

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According to the IWCLL guidelines the following conditions define active disease: significant B-symptoms, cytopenias not caused by autoimmune phenomena, autoimmune anemia and/or thrombocytopenia poorly responsive to conventional therapy, symptoms or complications from lymphadenopathy, splenomegaly or hepatomegaly as well as lymphocyte doubling time of less than 6 months (only in patients with more than 30 G lymphocytes/L) [16]. These criteria may also lead to treatment initiation in early stage disease. The absolute lymphocyte count itself should not be used as a sole indicator for treatment initiation, because even patients with high lymphocyte counts may remain symptom-free.

6.3 General Considerations for the Choice of Initial Therapy

Because of the broad spectrum of available therapies for CLL, the selection of the optimal initial treatment has become complex. Among those parameters, which have to be considered for treatment recommendation, are the following:

- Fitness and comorbidity burden
- Genetic risk of the leukemia
- Comedication

As outlined above, treatment should only be administered in patients with advanced and/or symptomatic disease.

Because CLL is a disease of elderly patients with a median age of 72 years at diagnosis [17], the evaluation of the patients' fitness and comorbidity burden is very important. For this purpose no ideal fitness stratification tool exists so far. The performance status (Eastern Cooperative Oncology Group (ECOG) status or Karnofsky index) is of limited evidence in CLL and can therefore not be used as a single tool for a reliable guide to treatment. Therefore, it is recommended to add another tool for measuring patients' comorbidity burden. In solid tumors and lymphoma there are several tools available, among these the Cumulative Illness Rating Scale (CIRS) [18, 19], Charlson Comorbidity Index (CCI) [20], and National Cancer Institute Comorbidity Index [21]. A comprehensive geriatric assessment measuring dimensions of aging in addition to performance status and comorbidities would be beneficial for the best treatment choice [22, 23].

Based on their physical constitution, comorbidities, and estimated life expectancy, regardless of their specific cancer diagnosis, three groups of elderly cancer patients can be distinguished [24–26]. First, physically fit patients without, or with only mild, comorbidities that do not adversely impact on their life expectancy should be treated with standard therapies. Second, patients with relevant comorbidities that have an impact on life expectancy should receive dose-reduced or modified therapies for disease control. Third, patients with a markedly reduced life expectancy due to multiple and/or severe comorbidities or frailty should be treated with best supportive care. For these three patient groups there are different therapeutic goals: the aim for the first group is to achieve a long-term remission and a prolongation of survival, whereas for the second and third group disease control and symptom control/palliation should be sought.

Another important factor for the choice of treatment is the genetic risk profile of CLL. The deletion of the short arm of chromosome 17 [del(17p)], or mutation of the *TP53* gene, is associated with a poor prognosis and resistance to chemotherapeutic agents [27–31]. Therefore, the detection of a del(17p) or a *TP53* mutation is crucial for the choice of treatment and a genetic analysis (FISH and molecular testing for del(17p) and *TP53* mutation) is strongly recommended before treatment initiation [30] (see Chap. 7). Because of the possibility of genetic evolution [32–34] testing for specific genetic markers should be repeated before treatment initiation if the previous testing was done more than 6 months ago. The likely occurrence of a new genetic mutation or deletion should particularly be considered in those patients in whom the clinical course of the disease has changed to a more aggressive form.

Besides these most relevant factors driving the treatment decision there are some other factors that may influence the choice of treatment.

Among them is the patients' expected compliance, which is essential for CLL treatment with oral substances such as kinase inhibitors. Moreover, with the new continuous treatments, such as ibrutinib, potential interactions with comedication will also have to be considered.

6.4 Treatment of Fit Patients

6.4.1 Purine Analogue Based Chemoimmunotherapies

Combinations of CD20 antibodies with fludarabine-based chemotherapeutic backbone are the most intensive chemoimmunotherapeutic options in CLL resulting in high rates of MRD negativity. Table 6.1 summarizes a selection of trials evaluating these combinations.

6.4.1.1 Combination Therapies with Rituximab

After preclinical studies showed a synergy between fludarabine and rituximab [49], fludarabine-based chemoimmunotherapy combinations were evaluated in phase II trials [50]. A randomized CALGB (Cancer and Leukemia Group B) study, CALGB 9712, compared the efficacy of the concurrent administration of rituximab and fludarabine (FR) versus fludarabine alone in 104 previously untreated CLL patients [35] (Table 6.1). In both arms induction therapy was followed 2 months later by four weekly doses of rituximab for consolidation therapy. The trial showed a significant

Table 6.1 Efficacy of selected chemoimmunotherapies in frontline CLL for physically fit and non-comorbid patients

Reference and study design	No. of patients	Treatment regimen	Clinical response		Progression-free survival	Overall survival
			CR	CR + PR		
Fludarabine + rituximab (FR)						
Byrd et al. [35, 36] Phase II randomized	51	F 25 mg/m^2 d1–5 iv q 28 days × 6 R 375 mg/m^2 d1,4 C1 and d1 C2–6 R 375 mg/m^2 × 4 for consolidation	47%	90%	67% at 2 years (median 42 mo for both groups)	93% at 2 years (median 85 months for both groups)
Fludarabine, cyclophosphamide + rituximab full dosed (FCR)						
Keating et al. [37, 38] Phase II	224	F 25 mg/m^2 d1–3 iv q 28 days × 6 C 250 mg/m^2 d1–3 iv q 28 days × 6 R 375 mg/m^2 d1 C1 and R 500 mg/m^2 d1 C2–6	70%	95%	Median 6.4 years	Median 12.7 years
[39–41] Phase III	408	F 25 mg/m^2 d1–3 iv q 28 days × 6 C 250 mg/m^2 d1–3 iv q 28 days × 6 R 375 mg/m^2 d1 C1 and R 500 mg/m^2 d1 C2–6	44%	93%	Median 57 months	79% at 5 years
Pentostatin, cyclophosphamide + rituximab (PCR)						
Kay et al. [42] Phase II	65	P 2 mg/m^2 d1 q 21 days × 6 CYC 600 mg/m^2 d1 q 21 days × 6 R 375 mg/m^2 d1 q 21 days × 6	41%	91%	Median 33 months	n.a.

(continued)

Table 6.1 (continued)

Reference and study design	No. of patients	Treatment regimen	Clinical response		Progression-free survival	Overall survival
			CR	CR + PR		
Reynolds et al. [43] Phase III	92[a]	P 4 mg/m^2 d1 q 21 days × 6 CYC 600 mg/m^2 d1 q 21 days × 6 R 375 mg/m^2 d1 q 21 days × 6	7%	49%	63% at 2 years	79% at 2 years
Fludarabine, cyclophosphamide + ofatumumab (FCO)						
Wierda et al. [44] Phase II randomized	61	F 25 mg/m^2 d1–3 q 28 days × 6 CYC 250 mg/m^2 d 1–3 q 28 days × 6 O Rando 1: 300 mg d1 C1, 500 mg i.v d1 C2–C6 q 28 days × 6 O Rando 2: 300 mg d1 C1, 1000 mg i.v d1 C2–C6 q 28 days × 6	Rando 1: 32% Rando 2: 50%	Rando 1: 77% Rando 2: 73%	n.a. (after median observation time of 8 months 70% at 2 years)	n.a. (after median observation time of 8 months >90% at 2 years)
Pentostatin, cyclophosphamide + ofatumumab (PCO)						
Shanafelt et al. [45] Phase II	48	P 2 mg/m^2 d1 q 21 days × 6 CYC 600 mg/m^2 d1 q 21 days × 6 O 300 mg d1 C1, 1000 mg d2 C1 O 1000 mg d1 q 21 days × 6	46%	96%	n.a.	n.a.
Bendamustine + rituximab (BR)						
Fischer et al. [46] Phase II	117	B 90 mg/m^2 d1 + 2 q 28 × 6 R 375 mg/m^2 d1 C1 and R 500 mg/m^2 d1 C2–6	23%	88%	Median 34 months (event free survival)	At 2 years 90%
Eichhorst et al. [47] Phase III	279	B 90 mg/m^2 d1 + 2 q 28 × 6 R 375 mg/m^2 d1 C1 and R 500 mg/m^2 d1 C2–6	31%	98%	Median 42 months	At 3 years 92%
Bendamustine + ofatumumab (BO)						
Flinn et al. [48]	43	B 90 mg/m^2 d1 + 2 q 28 × 6 O 300 mg d1 C1, 1000 mg d8 C1 O 1000 mg d1 C2–6	43%	96%	Median PFS not reached at 29 months	n.a.

All agents were given intravenously unless otherwise specified
F fludarabine, *CYC* cyclophosphamide, *P* pentostatin, *R* rituximab, *O* ofatumumab, *B* bendamustine, *d* day, *C* cycle, *mo* months, *n.a.* not available
[a]Including 19 patients with prior chemotherapy

benefit of the concurrent administration even with long-term follow-up [35, 36].

The triple combination of fludarabine, cyclophosphamide, and rituximab (FCR) was initially investigated in a phase II trial performed at the MD Anderson Center in frontline therapy of patients with advanced CLL [37] (Table 6.1). Long-term follow-up after almost 13 years showed a median PFS of 6.4 years and a median OS of 12.7 years [38].

The CLL8 trial of GCLLSG compared the FCR regimen head-to-head with the FC regimen [39] (Table 6.1). Eight hundred and seventeen patients in good physical fitness, defined as CIRS comorbidity score ≤6 and a creatinine clearance >70 ml/min, were randomly assigned to receive six courses of FC or FCR. FCR induced a higher overall response rate than FC (93% versus 85%; $p < 0.001$), more complete responses (44% versus 23%; $p < 0.001$), and a significantly higher proportion of minimal residual disease (MRD) negativity at the end of treatment (63% versus 35%, $p < 0.001$) [40]. PFS at 2 years was 77% in the FCR arm and 62% in the FC arm ($p < 0.01$) [39]. An update of the CLL8 data after a median observation time of 5.9 years confirmed the overall survival benefit with FCR first-line therapy (Table 6.1) [41]. Interestingly, the median PFS was still not reached in FCR-treated patients with mutated IGHV status. Their Kaplan--Meier PFS curve appeared to level off to a plateau, raising expectations that a subgroup of patients treated with FCR might not only achieve long-term remissions but may also be cured of the disease [41].

However, FCR is associated with a high incidence of adverse events, particularly cytopenias and infections. In the CLL8-trial CTC°III–IV leucopenias and neutropenias occurred in 24% and 34% of patients treated with FCR and 25% experienced CTC°III–IV infections [39]. Though neutropenias were significantly more frequent than with FC, the incidence rate of severe infections was similar in both arms.

Several studies investigated the addition of other compounds to FCR or FR, such as an additional chemotherapeutic agent or an immunomodulatory drug, in order to increase response quality. A Spanish multicenter phase II trial evaluated the addition of mitoxantrone to FCR and showed no clear benefit [51]. A British randomized phase II trial comparing FCR versus FCR with mitoxantrone (FCMR) showed similar CR rates (70% for FCR and 69% for FCMR) as well as similar MRD negativity rates (59% versus 50%) in both arms [52].

A phase I study evaluating the addition of lenalidomide to fludarabine and rituximab (FRL) as first-line therapy had to be stopped early because of high toxicity rates in nine patients [53].

An Austrian phase II study was more successful, when [54] lenalidomide was started with a delay of 1 week on the same dose level of 2.5 mg with a planned increase to 25 mg [54]. Maintenance therapy with lenalidomide for 6 months with three additional rituximab infusions was administered after six courses of FRL. Severe neutropenia developed in 27%, but severe infections developed in 5% only. Though improvement of response from partial remission after induction to complete remission at the end of maintenance was observed in 25% of the patients, many patients required dose reduction due to severe neutropenia.

A recently published smaller study including 20 treatment naive patients reported successful outcomes with dose-reduced FCR (FCR light) regimen in combination with lenalidomide [55]. In this trial pegfilgrastim was given routinely with every cycle. The 75% complete remission rate with this regimen is promising. Severe neutropenia occurred in 52% of the patients, severe infection in 8%, and severe rash in 5%.

These results show that the administration of additional chemotherapeutic or immunomodulating substances to the FCR or FR regimen may increase the toxicity. However, ongoing studies are now investigating the addition of the Btk-inhibitor ibrutinib to FCR.

Other purine analogue based combinations substituted fludarabine within the FCR regimen with cladribine (CCR) or pentostatin (PCR). The combination of cladribine plus cyclophosphamide plus rituximab was assessed in patients with previously untreated CLL showing a 73% overall response rate with 22% com-

plete responses, which were slightly lower rates than with FCR frontline [56]. The combination of pentostatin, cyclophosphamide plus rituximab was evaluated in 65 previously untreated CLL patients [42] (Table 6.1). Response rates and toxicity rates were similar to fludarabine-based combination therapies. In a phase III randomized trial comparing FCR to PCR in previously untreated or minimally pretreated CLL patients, there were no statistical differences between treatments in OS or response [43] (Table 6.1). Also infection rates showed no differences between the arms.

A phase II study evaluating the combination of pentostatin and rituximab (PR) without cyclophosphamide yielded only a 76% overall response rate and a 27% complete response rate. In addition, relapse-free survival was longer with PCR as compared to PR in a historical control; thus, the authors concluded that the addition of cyclophosphamide to purine-analogue base chemoimmunotherapy is necessary for a better remission induction [57]. Although no data regarding a head-to-head comparison between FCR and FR are available, in most countries and regions FCR has become the standard therapy in fit patients needing initial therapy. However, as there might be a potentially higher risk for secondary neoplasias after initial therapy with FCR [58], in some areas the FR combination is preferred [59].

6.4.1.2 Purine Analogues Based Combinations with Ofatumumab

The fully humanized CD20 antibody ofatumumab was also tested in several studies with purine analogue based combinations. An international phase II trial evaluated two different dose levels of ofatumumab combined with fludarabine and cyclophosphamide (FCO) as frontline therapy for CLL. Sixty-one patients were randomized between two different ofatumumab doses (500 mg versus 1000 mg) [44]. The toxicity profile was very similar to FCR (severe neutropenia in 48%, thrombocytopenia in 15%, anemia in 13%, and infection in 8%) [44] (Table 6.1). The lower overall response rate of FCO in comparison to FCR might have been related to the higher-risk profile of the patients included.

Another phase II study investigated the combination of ofatumumab, pentostatin, and cyclophosphamide (PCO) in 48 previously untreated CLL patients [45] (Table 6.1). Response rates and a 79% PFS at 24 months were comparable with the response rates of historical trials of rituximab-based chemoimmunotherapy.

6.4.1.3 Purine Analogue Based Combinations with Other Antibodies

Since 2012 the CD52antibody alemtuzumab (A) has no longer been easily accessible for CLL treatment as the license was withdrawn by the manufacturer. Previous studies evaluating combination therapies of fludarabine plus alemtuzumab have shown a high efficacy rate, but also a high toxicity rate, particularly regarding infections [60, 61].

The phase III trial of the French study group, which compared FCA to FCR in first-line therapy, was closed prematurely due to the higher toxicity and treatment-related mortality observed in the FCA arm [62]. In this trial, alemtuzumab was given subcutaneously. The therapeutic efficacy of FCR was clearly superior to FCA with a 3-year PFS of 83% with FCR and 72% with FCA [62].

Another international phase III study conducted by the Nordic and the HOVON group compared FC versus FCA using alemtuzumab in a significantly lower dose than the French study [63]. The study showed that FCA prolonged progression-free survival (3 year PFS 53% versus 37%), which was the primary end point, but did not impact OS [63]. Opportunistic infections were more frequent following FCA, but without an increase in treatment-related mortality.

In a phase II study at the MD Anderson Center alemtuzumab was added to FCR (CFAR) in 60 high-risk untreated patients [64]. Complete remission was achieved in 70% and partial remission in 22% resulting in an overall response of 92%. Of 14 patients with a del(17p) eight (57%) achieved a complete response. The median PFS was 38 months and median OS was not reached.

6.4.2 Bendamustine Based Combinations with CD20 Antibodies

The alkylating agent bendamustine was combined with rituximab in 117 patients with previously untreated CLL [46] (Table 6.1). The overall response rate was 88% with a complete response rate of 23%. Severe neutropenia and infections were not frequent (20% and 8% of patients) [46]. A phase III trial of the GCLLSG (CLL10) therefore prospectively evaluated whether the BR-regimen was indeed equally effective and less toxic compared to the current standard treatment, FCR, for the first-line treatment of physically fit patients. Five hundred and sixty-four physically fit patients without del(17p) were randomized to receive up to 6 cycles of either FCR or BR [47] (Table 6.1). Overall response rates were 98% in both arms, but patients treated with FCR achieved a significantly higher rate of complete remissions (41% versus 31%) and a longer median PFS (55.2 vs. 41.7 months, HR = 1.643, 95% CI 1.308–2.064, p = 0.0003). Despite these PFS differences no difference in OS was observed. On the other hand significantly more common toxicity criteria (CTC) grade three and four neutropenias and infections occurred with FCR (88% versus 68% and 40% versus 25%), particularly in patients >65 years old (48% versus 27%) [47]. Taken together, based on the CLL10 study FCR remains the standard therapy in very fit CLL patients, but due to the lack of OS difference and the toxicity profile, elderly fit CLL patients might benefit from BR as an alternative regimen.

The combination of bendamustine plus ofatumumab (BO) was investigated in previously untreated and relapsed CLL within a small phase II study. The investigator-assessed overall and complete response rates were 95% and 43% in the 44 patients, including those receiving BO as first-line treatment [48] (Table 6.1). CTC°III/IV adverse events occurred in 57% of first-line patients, including 36% CTC°III/IV neutropenias and 11% CTC°III/IV infections [48]. Based on the results of this phase II trial the combination of BO is approved for use in CLL.

In conclusion these data show that BR is inferior to FCR with regard to efficacy but is better tolerated. Because of the lower rate of severe infections BR/BO frontline treatment might be considered in those fit CLL patients with a high risk of infectious episodes.

6.5 Treatment of Less Fit Patients

6.5.1 Chlorambucil-Based Combinations

In contrast to the progress made in younger CLL patients by intensifying the treatment given, in elderly/comorbid patients single agent chlorambucil was until recently still widely used, because no statistically significant differences in OS were found [65–67]. In order to study the effects of intensifying the chlorambucil regimen several studies evaluated the combination of chlorambucil with different CD20-antibodies.

In a first step, two phase II trials assessed the combination of chlorambucil plus rituximab (ClbR) in an elderly patient population [68, 69]. In the British study 100 patients ineligible for fludarabine-based treatment received 6 cycles of ClbR [68] (Table 6.2). In the Italian study 85 patients older than 65 years were included [69] (Table 6.2), treated with 6 cycles of ClbR and afterwards randomized to 2 years of rituximab maintenance therapy versus observation. Median PFS was 23.5 months in the British trial and 34.7 months in the Italian trial [69]. The combination ClbR was well tolerated in both clinical studies with severe infections occurring in less than 10% of the patients.

Obinutuzumab is a humanized and glycoengineered CD20 antibody, which showed in vitro increased direct cell killing and antibody-dependent cellular cytotoxicity (ADCC) [77] as well as high rates of apoptosis in comparison to rituximab [78, 79]. The addition of rituximab or obinutuzumab to chlorambucil was tested in the CLL11 trial, an international phase III study [70] (Table 6.2). Seven hundred and eighty-one patients with coexisting medical conditions

Table 6.2 Efficacy of selected chemoimmunotherapies in frontline CLL for less fit and comorbid patients

Reference and study design	No. of patients	Treatment regimen	Clinical response		Progression-free survival	Overall survival
			CR	CR + PR		
Chlorambucil + rituximab (ClbR)						
Hillmen et al. [68] Phase II	100	CLB 10 mg/m² d 1–7q 28 × 6 (additional 6 cycles CLB mono in patients not in CR) R 375 mg/m² d 1 C1 and 500 mg/m² d1 C2–C6	10%	84%	Median 23.5 months	At 30 months 84%
Foa et al. [69] Phase II randomized	85	CLB 8 mg/m² d 1–7 R 375 mg/m² d1 C3 and 500 mg/m² d1 C4–C8 Responders were randomized: R 375 mg/m² q56d × 12 or observation	19%	82%	Median 34.7 months	Median not reached
Goede et al. [70] Phase III randomized	330	CLB 0.5 mg/kg BW d1 + 15 q28 × 6 R 375 mg/m² d 1 C1 and 500 mg/m² d1 C2–C6	7%	66%	Median 15.2 months	Median not reached
Chlorambucil + obinutuzumab (G-Clb)						
Goede et al. [70] Phase III randomized	333	CLB 0.5 mg/kg BW d1 + 15 q28 × 6 Obinutuzumab 1000 mg d1,8,15 C1 and 1000 mg d1 C2–C6	22%	77%	Median 26.7 months	Median not reached
Chlorambucil + Ofatumumab (ClbO)						
Hillmen et al. [71]	221	Clb 10 mg/m² d1–7 q 28 days OC1 d1 300 mg, d8 1000 mg, C 2–12 d1 1000 mg q 28 days	14%	82%	Median 22.4 months	At 3 years 85%
Fludarabine, cyclophosphamide + rituximab dose-reduced (FCR light)						
Foon et al. [72, 73] Phase II	65	F 20 mg/m² d2–4 C1 and d1–3 q 28 days × 6 C2–5 CYC 150 mg/m² d 2–4 C1 and d1–3 q 28 days × 6 C2–5 R 375 mg/m² d1 C1 R 500 mg/m² d1+ d14 q 28 days C2–6 R maintenance 500 mg/m² q3 months	73%	94%	Median 68 months	Not yet reached
Mulligan et al. [74] Phase II randomized	41	FCR3 F 24 mg/m² p.o. d1–3 q 28 days × 6 CYC 150 mg/m² p.o. d1–3 q 28 days × 6 R 375 mg/m² i.v.d1 C1 R 500 mg/m² i.v.d1 q 28 C2–6	51%	95%	75% at 18 months	90% at 18 months
Mulligan et al. [74] Phase II randomized	38	FCR5 F 24 mg/m² p.o. d1–5 q 28 days × 6 CYC 150 mg/m² p.o. d1–5 q 28 days × 6 R 375 mg/m² i.v. d1 C1 R 500 mg/m² i.v. d1 C2–5 q 28 days	79%	97%	65% at 18 months	83% at 18 months

(continued)

Table 6.2 (continued)

Reference and study design	No. of patients	Treatment regimen	Clinical response		Progression-free survival	Overall survival
			CR	CR + PR		
Dartigeas et al. [75] Phase III randomized	194	F 40 mg/m^2 p.o. d1–3 q 28 days × 4 CYC 250 mg/m^2 p.o. d1–3 q 28 days × 4 R 375 mg/m^2 i.v. d1 C 1 500 mg/m^2 d14 C1, d1 + 14 C2, d1 C 3&4	20%	96%	n.a.	n.a.
Bendamustine + rituximab (BR)						
Michallet et al. [76] Phase IIIb	121	B 90 mg/m^2 d1 + 2 q 28 × 6 R 375 mg/m^2 d1 C1 and R 500 mg/m^2 d1 C2–6	24%	91%	Median 40 months	n.a.

All agents were given intravenously unless otherwise specified
Clb chlorambucil, *R* rituximab, *O* ofatumumab, *G GA101* obinutuzumab, *F* fludarabine, *CYC* cyclophosphamide, *B* bendamustine, *d* day, *C* cycle, *mo* months, *n.a.* not available

(defined as a CIRS Score >6 and/or creatinine clearance <70 ml/min) were randomized to receive single agent chlorambucil (Clb) or Clb with either rituximab (ClbR) or obinutuzumab (ClbG). The incidence of infusion-related reactions (IRR) in general and especially severe IRRs was higher with ClbG than with ClbR (CTC°I–IV and °III–IV: 66% and 20% vs. 38% and 4%). However, with the introduction of safety precautions for prevention of IRRs, such as adequate premedication, dose-splitting of the first dosage, and withholding antihypertensive medications, these were manageable and limited to the first administration of obinutuzumab. Also, cytopenias, especially neutropenias, were more common with ClbG and ClbR compared to single agent chlorambucil (neutropenia CTC°III–IV: 33% and 28% vs. 10%) but did not lead to a higher rate of infections (infection CTC°III–IV: 12%, 14%, and 14%) [70]. As expected, the overall and complete response rates were highest with ClbG (overall response rate 77.3%, including 22.3% complete responses), followed by ClbR (65.6% and 7.3%), and were inferior with single agent chlorambucil (31.4%, no complete responses). In the ClbG arm a significant proportion of responding patients even achieved MRD negativity in peripheral blood and bone marrow (37.6% and 19.5% of all evaluated patients) [70]. The median PFS was only 11.1 months in patients receiving single agent chlorambucil versus 16.3 months with ClbR ($p < 0.0001$) and 26.7 months with ClbG ($p < 0.0001$). ClbG also improved the median OS in comparison to single agent chlorambucil ($p = 0.0022$). An updated analysis showed that OS in the ClbR arm was also significantly improved in comparison to CLB alone (HR 0.60, 95% CI 0.38–0.94, $p = 0.0242$) [80]. The difference in OS between the two antibody-containing treatment arms was not statistically significant (HR 0.70, 95% CI 0.47–1.02, $p = 0.0632$).

The combination of chlorambucil and ofatumumab (ClbO) was compared to single agent chlorambucil in a total of 447 treatment-naïve CLL patient, who were considered unsuitable for fludarabine-based therapy due to their age or comorbidities [71] (Table 6.2). The overall and complete response rates were significantly higher with the addition of ofatumumab compared to chlorambucil alone (82% including 12% CRs vs. 69% including 1%, $p < 0.001$), including higher rates of MRD negativity (18% vs. 1%) which translated into an increase of 9 months in the median PFS (22.4 vs. 13.1 months, $p < 0.001$).

So far, no randomized head-to-head comparison between ofatumumab and other anti-CD20 antibodies has been performed. However, based on the data of these two phase III studies chlorambucil-based chemoimmunotherapy combinations are recommended for frontline therapy of elderly/comorbid patients without del(17p).

6.5.2 Purine Analogue Based Combinations

Because of the high incidence of toxicities associated with full dose FCR in elderly patients with relevant comorbidity, a dose-modified FCR-Lite regimen was designed to maintain the efficacy but decrease the toxicity of the FCR regimen [72, 73] (Table 6.2). Dosing of both cytostatic agents was reduced (fludarabine to 20 mg/m^2 and cyclophosphamide to 150 mg/m^2 for 3 days) and the dose of rituximab increased (administered on day 1 and day 14). In addition, maintenance with rituximab at 500 mg/m^2 was given every 3 months until progression. A high complete response rate of 73% resulted in a median PFS of 5.8 years. Grade 3/4 neutropenia was documented in only 11% of cycles and severe infection in 7%. Notably the median age of patients included in this study was only 58 years and therefore not representative of an elderly patient population.

Several other studies evaluated different dose-reduced FCR regimens. Mulligan et al. investigated two different dose-reduced FCR regimens and a FR regimen in 116 fit CLL patients 65 years of age or older [74] (Table 6.2). The FCR 3 and FCR 5 schedule consisted of three or 5 days, respectively, of fludarabine 24 mg/m^2 and cyclophosphamide 150 mg/m^2 orally administered in combination with rituximab given intravenously (375 mg/m^2 at first cycle and then 500 mg/m^2). The FR regimen (fludarabine 24 mg/m^2 d1–5) was associated with the lowest toxicity, but also yielded significantly lower complete remission rates than FCR3 and FCR5. Only 44% of the patients receiving FCR5 completed 6 cycles of therapy, which may account for the shorter PFS seen with FCR5 compared with FCR3 [74].

A French study evaluated an orally administered FCR-Lite schedule in fit CLL patients above the age of 65 years [75] (Table 6.2). Four cycles of dose-reduced FC p.o. were combined with intensified six doses of rituximab for induction treatment similar to the US study by Foon et al. [72]. After induction therapy patients achieving a partial remission were randomized between rituximab maintenance and observation. This dose-reduced FCR regimen was also well tolerated in the elderly and resulted in a promising overall response rate of 96% with 20% complete responses [75] (Table 6.2) [75].

The Czech CLL study group initiated an observational study, without age or fitness limits, in which 207 CLL patients were included [81]. One hundred and eight patients with a median age of 69 years and a median CIRS score of 5 received FCR-Lite with the FC dose reduced to 50% and full dose rituximab. Clinical complete remissions were achieved in 37%, but median PFS was only 28 months, which was significantly shorter than with full dose FCR [81].

Summarizing the results from these studies evaluating dose-reduced FCR the data show that the toxicity of this regimen is acceptable in elderly patients. The inferior efficacy might be compensated for by a higher dose of rituximab with the FCR-Lite regimen.

6.5.3 Bendamustine Based Combinations

Most of the clinical trials evaluating the combination of bendamustine plus rituximab did not focus particularly on elderly or less fit patients. Subgroup analyses of larger studies showed good response rates (overall response rate 84–96%; complete response rate 11–35%) and median time to progression of 38–48 months [46, 47] in elderly or less fit patients.

The Mable study investigated BR in a randomized phase IIIb study in comparison to ClbR in 241 elderly patients ineligible for frontline therapy with FCR as well as 116 patients at second line [76] (Table 6.2). The analysis showed a higher complete response rate with BR in comparison to ClbR at first line (24% versus 9%; $p = 0.002$). Median PFS was significantly extended in the BR arm in comparison to the ClbR group (40 vs. 30 months; $p = 0.003$), but no difference in OS was observed [76]. With regard to toxicities no significant differences in terms of hematotoxicity were observed (43% versus 37% severe neutropenia), but a tendency towards more infections, reported as SAEs, was observed with BR (19% versus 8%) [76].

6.6 Non-Chemotherapy Containing Frontline Therapy Options

Ibrutinib is a first-in-class orally available inhibitor of Bruton tyrosine kinase (BTK). This substance is given continuously and is only discontinued in the event of intolerable side effects or progression of CLL. Phase II trials in patients with relapsed/refractory CLL have yielded high response rates including in 17p-deleted cases [82–84]. In the frontline setting, the phase III RESONATE-2 trial showed superiority of ibrutinib over chlorambucil in 269 elderly, untreated CLL patients. Ibrutinib was superior to chlorambucil with regard to ORR (86% vs. 35%), median PFS (not reached vs. 18.9 months), and 2-year-OS (98% vs. 85%) [85] (Table 6.3). Based on these trials, ibrutinib was approved for therapy of treatment-naïve as well as pretreated CLL patients, including patients with 17p deletion.

Data on longer follow-up with ibrutinib frontline therapy are available from 31 elderly patients treated within a phase II trial [85]. After a median observation time of 5 years, overall response was 80% with 29% complete responses. The 5-year progression-free survival rate in these patients was 91%. While 55% of the patients are still under ibrutinib therapy, 45% stopped mainly due to side effects [85]. Though most side effects occurring with ibrutinib are mild or disappear after the first years of treatment, reported gastrointestinal side effects, hypertension, exanthema, and arthritis may lead to cessation of treatment, as may artitrial fibrillation, which is reported in up to 14% of CLL patients [86]. Newer BTK inhibitors, which will be available soon, might have a more favorable side effects profile, at least with respect to the incidence of atrial fibrillation [87].

Ibrutinib has been compared to more intensive chemoimmunotherapies, such as BR or CLb + Obinutuzumab, within randomized trials, showing superiority with respecte to PFS, but not OS [88, 89]. Trials which are currently recruiting, such as the FLAIR trial of the UK study group, will show whether chemotherapy-free options are superior to conventional chemoimmunotherapy and which treatment is most suitable for different patient subgroups. Due to the fact that unmutated IGHV status did not result in a shorter PFS under treatment with ibrutinib in comparison to mutated IGHV, treatment with a BTK inhibitor might be considered particularly in patients with unmutated IGHV status.

With the approval of ibrutinib in frontline CLL without TP53 mutation/deletion, some other non-chemotherapeutic approaches now play a less important role. Rituximab administered either as monotherapy or in combination with steroids for treatment of elderly patients is a frequently used frontline regimen in the USA [90, 91].

The immunomodulatory agent lenalidomide, which is not approved in CLL, showed a relatively low 65% overall response rate, [92] but with long lasting remissions >36 months in 58% of the patients after long-term observation [93]. However, a randomized phase III study comparing chlorambucil versus lenalidomide in frontline therapy of elderly patients was terminated early due to an excess of deaths in the lenalidomide arm, though this was not observed with longer follow-up [94].

Table 6.3 Efficacy of ibrutinib in frontline CLL

Reference and study design	No. of patients	Treatment regimen	Clinical response		Progression-free survival	Overall survival
			CR	CR + PR		
Ibrutinib						
Burger et al. [85] Phase III randomized	269	Ibrutinib 420 mg daily until progression	4%	86%	Median not reached	At 2 years: 98%

6.7 Outlook: New Combinations

Several phase II and phase III trials are currently investigating chemotherapy-free combinations in the relapsed situation as well as in frontline. In particular, combinations based on venetoclax, a *BCL2* inhibitor, show promising data with regard to deep responses, including high rates of MRD-negativity [95, 96].

Within a safety run-in phase Ib trial the combination of obinutuzumab with venetoclax, added 3 weeks after the start of obinutuzumab, was administered in 13 comorbid CLL patients for a total of 6 months, followed by 6 additional months of monotherapy with venetoclax [97]. The treatment was well tolerated, and no clinical tumor lysis syndrome occurred though the majority of the patients had decreased renal function. All patients responded, 58% had a complete response and 11 of 12 assessed patients were MRD negative in the peripheral blood [97]. A similar high rate of MRD negativity was reported in a phase II trial including 34 patients who were not previously treated [98]. Thirty-two of thirty-four patients received one to two courses of debulking with bendamustine before treatment with obinutuzumab plus venetoclax as induction therapy for 6 months, followed by venetoclax and obinutuzumab maintenance until MRD negativity was confirmed. In an early analysis after induction therapy 91% of the patients had no detectable CLL cells in the peripheral blood [98]. A randomized controlled trial enrolled 420 comorbid patients who were randomized between chemoimmunotherapy with chlorambucil plus obinutuzumab versus venetoclax plus obinutuzumab. Results are expected in 2019.

A combination therapy consisting of ibrutinib plus venetoclax, without a CD20 antibody, was evaluated in a phase II trial in relapsed CLL [99]. After 8 weeks of ibrutinib monotherapy venetoclax was added and gradually ramped up. Fifty patients have been treated so far, showing good tolerance of this regimen. In spite of the presence of high-risk factors all 25 evaluable patients responded; 60% had a complete response and 76% had less than 1% CLL cells in the bone marrow [99]. Due to these promising results the venetoclax plus ibrutinib combination is now being evaluated as an additional treatment arm within the FLAIR trial of UK CLL Study Group.

Among the chemotherapy-free combinations the addition of the CD20 antibody rituximab to Btk-inhibitors appears to have only minor benefits. A randomized trial in relapsed CLL patients as well as in patients with high-risk genetic features requiring frontline therapy compared ibrutinib alone versus ibrutinib plus 6 cycles of rituximab [100]. The results showed no significant differences in response rates and progression-free survival. Currently, ongoing trials are investigating ibrutinib plus obinutuzumab as well as the triple combination ibrutinib, venetoclax, and obinutuzumab.

6.8 Conclusion

Treatment decisions in CLL have become very complex. Besides clinical stage, the patient's prognostic risk profile and comorbidities have to be considered for the choice of therapy. In general, the most efficacious CLL treatment should be administered upfront, because a survival benefit has been demonstrated with chemoimmunotherapies (FCR, ClbG; ClbR) used in the first-line setting [39, 70, 80] (Fig. 6.1). For patients without very high-risk genomic alterations first-line treatment with chemoimmunotherapy is a well examined and established standard of care. FCR is the standard treatment in physically fit patients with mild or no comorbidities. Depending on the burden of comorbidities less intense chemoimmunotherapy regimens based on bendamustine or chlorambucil in combination with CD20 antibodies should be used upfront in patients with relevant concomitant diseases. Independently from the type of chemoimmunotherapy regimen administered MRD negativity at the end of these treatment regimens is a strong predictor for long progression-free survival [101]. Patients with high-risk genomic alterations should be treated with the new targeted drugs whenever access is possible or within clinical studies (see Chap. 7). In the absence of high-risk genomic alterations the potential for achieving long-term remissions can guide the choice of frontline therapy in patients with mutated IGHV status [38]. In patients with unmutated IGHV status the disadvantages of chemoimmunotherapy, such as secondary

Fig. 6.1 First line therapy for chronic lymphocytic leukemia. CLL therapy is constantly changing. Based on novel findings that have been reported between the final submissions of the manuscript and the proof reading, the recommendations have been up dated

CLL first line treatment 2019

Stage	Fitness	del(17p) or p53mut	IGVH	Therapy
Binet A-B, Rai 0-II, inactive disease	Irrelevant	Irrelevant	irrelevant	None
Active disease or Binet C or Rai III- IV	Go go	No	M	FCR (BR above 65 years) or ibrutinib*
			U	Ibrutinib or FCR (BR above 65 years)*
		Yes	irrelevant	Ibrutinib, venetoclax or Idelalisib+Rituximab (if contraindications for ibrutinib)
	Slow go	No	M	Chlorambucil + Obinutuzumab or Ibrutinib*
			U	Ibrutinib or Chlorambucil + Obinutuzumab*
		Yes	irrelevant	Ibrutinib or Venetoclax (+ Obinutuzumab) or Idelalisib+Rituximab (if contraindications for ibrutinib)

*Consider and discuss with patient: long-term vs. fixed (6 m) duration therapy; lack of convincing evidence of overall survival differences; specific side effects of each therapeutic option: myelosuppression, infections, potential of secondary malignancies for CIT; Cardiac toxicity, bleeding and autoimmune disease for Ibrutinib.

CLL 2L treatment 2019

Response to 1L Therapy	Fitness	Therapy*
Refractory or progress within 3 years	Go go	Change to one of the following: Ibrutinib, Idelalisib + R, Venetoclax (+Rituximab). Additional options are FCR (after BR), A or A-Dex**, Lenalidomide (+R), BR (after FCR). Discuss consolidation with allogeneic SCT.
	Slow go	Change to one of the following: Ibrutinib, Idelalisib + R, Venetoclax (+Rituximab). Additional options are A or A-Dex**, FCR-lite, BR, Lenalidomide (+R), Ofatumumab**, HD Rituximab.
Progress after 3 years	All	Repetition of 1L therapy is possible.

* Recommendations are based on evidence, not approval or availability in the market.

** Alemtuzumab (A) or Ofatumumab are no longer marketed but may be available through compassionate use programs.

Stage	**Fitness**	**del(17p) p53mut**	**Therapy**
Rai 0-II/Binet A-B and inactive	irrelevant	irrelevant	watch & wait
Rai 0-II/Binet A-B and active or Rai III&IV/Binet C	Fit & low comorbidity burden	no	FCR* (>65y BR)
		yes	Ibrutinib (Idelalisib + R) discuss allo-SCT
	Less fit &/or significant comorbidity burden	no	CLB + anti-CD20 antibody, Ibrutinib
		yes	Ibrutinib (Venetoclax, Idelalisib + R)

malignancies or the development of chemo-resistant clones [102], have to be weighed against its possible benefits, such as the short period of treatment with chemoimmunotherapy compared with the continuous therapy required with novel drugs. The fact, that several novel agents are available in relapsed CLL after kinase inhibitor frontline therapy and no cross resistance has been demonstrated between classes of agents [103–105], supports the use of novel therapeutics in frontline of CLL with unfavorable genetic results.

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CLL with Del (17p)/TP53 Mutation

7

Eugen Tausch and Stephan Stilgenbauer

7.1 Introduction

Many prognostic factors have been established in chronic lymphocytic leukemia (CLL) in recent years, but only a few have found their way into daily clinical practice. Among these, chromosomal aberrations, specific gene mutations, and IGHV mutation status affect clinical outcome and therefore predict the course of the disease better than many previously identified clinical or laboratory parameters. Although CLL is characterized by a stable genome with a low number of mutations and aberrations in comparison to other malignancies, different recurrent copy number variations are present in more than 80% of CLL patients. As co-occurrence of aberrations is frequent, the establishment of a hierarchical system in the year 2000 was a milestone which translated the genomic fingerprint into a prognostic value [1]. Most common chromosomal aberrations comprise the deletion of chromosomes 13q, 11q, 17p, and 6q and gain of chromosome 12, while deletion of 17p (del17p) is a marker of the strongest adverse prognostic impact (Fig. 7.1a and b). This aberration involves the TP53 gene locus, coding for the 53 kDa protein *p53,* resulting in a loss of heterozygosity. Although chromosomal banding is still a valid method to determine the cytogenetic state of CLL, the development of hybridization arrays (CGH) and fluorescent microscopy expanded the available techniques for detecting 17p deletions. The current guidelines recommend fluorescent in situ hybridization (FISH) of metaphase or interphase chromosomes as the gold standard for a short turnaround time and inexpensive analysis, with a detection threshold of about 10% of affected cells (Fig. 7.2a). TP53 is affected not only by deletions but also point mutations. For TP53 mutation analysis even more methods are accessible including Sanger sequencing (Fig. 7.2b), functional assays of transactivation measurement (FASAY), and amplicon or exome enrichment-based next generation sequencing (NGS) approaches. Although TP53 mutations mainly locate in the coding region of the DNA binding domain coded by exons 4–8, mutations in exons 9 and 10 are well described and should be investigated following current ERIC recommendations [3].

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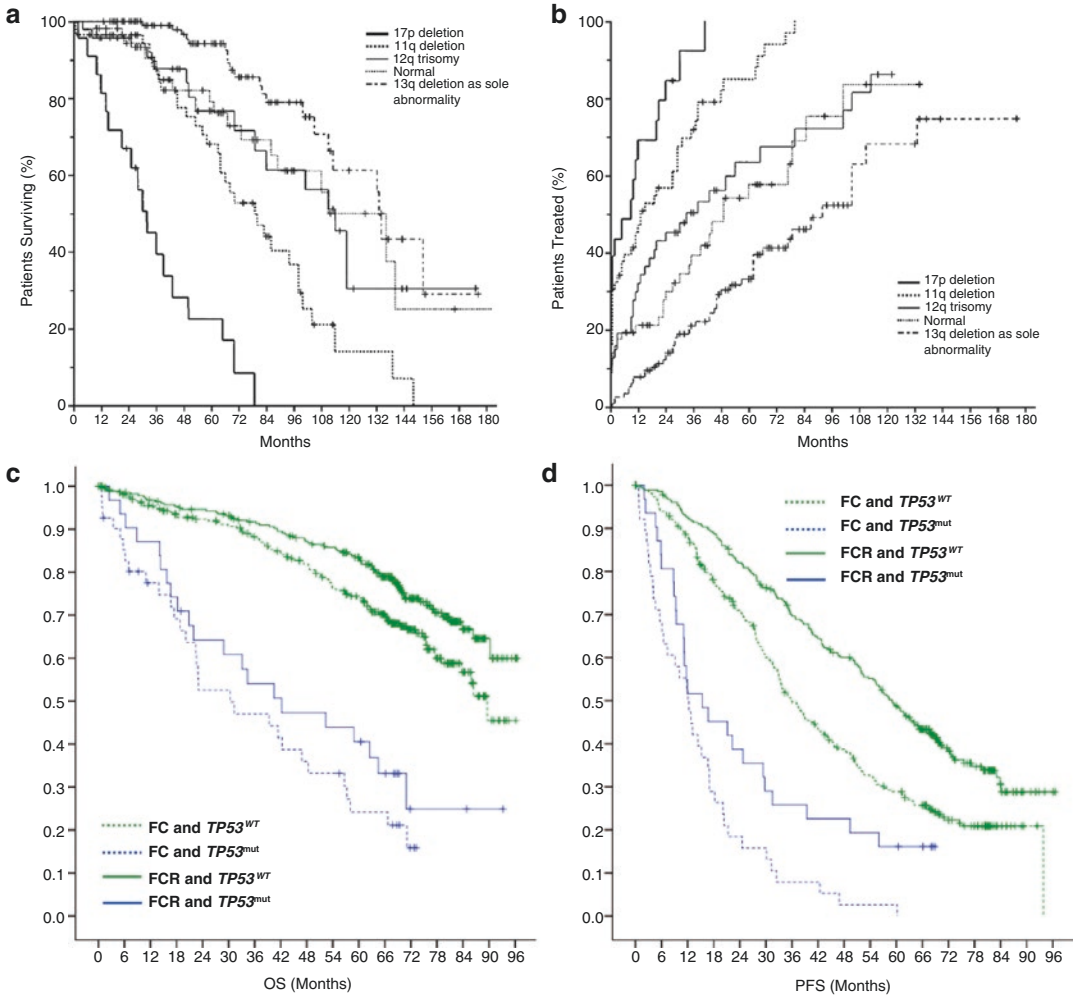

Fig. 7.1 Hierarchical model of recurrent genomic aberrations in CLL: probability of overall survival (**a**) from the date of diagnosis and of disease progression (**b**) as indicated by the treatment-free interval in patients from five genetic subgroups [1]. TP53 mutation status and impact on overall survival (**c**) and progression-free survival (**d**) in the CLL8 trial of the GCLLSG [2]

7.2 TP53: Oncogene or Tumor Suppressor

Discovered in 1979 by several groups [4–6], p53 was initially suspected to be an oncogene induced by leukemia-causing viruses such as simian virus 40. Different malignant cells were shown to express high levels of p53 and their proliferation correlated with the extent of p53 overproduction. Additionally fibroblasts were altered to immortalized morphologically changed tumorigenic cells in the presence of p53 [7, 8]. However Finlay et al. showed that most transformed primary rat embryo fibroblasts either failed to express p53 or expressed mutant p53 protein, implying a protective effect of functional p53 against malignant transformation [9]. As a result p53 has been identified as a tumor suppressor gene since 1979. Mice with homozygous inactivation of TP53 were shown to develop normally from murine embryonic stem cells, but then quickly obtained different tumors in a short time period [10]—a phenomenon also observed in a

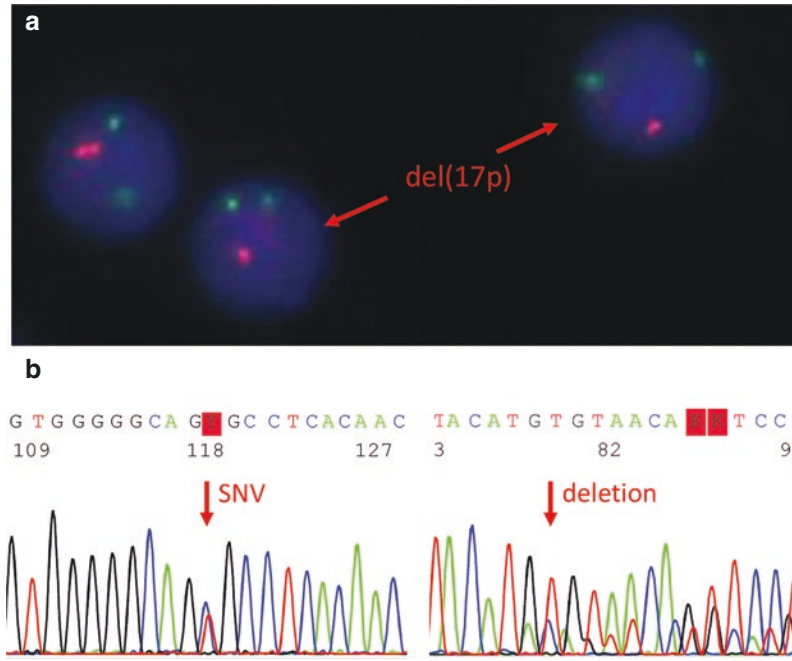

Fig. 7.2 (**a**) FISH analysis of a 17p deletion. Two of three depicted cells show only one 17p signal (red), but two 11q signals (green). (**b**) Nucleotide sequence of two different exonic spots in TP53 as examples of different gene mutations. Example 1 (left) shows an exchange of one base pair, here a cytosine to a thymidine, which results in an alternative amino acid sequence. Example 2 (right) shows a deletion of several bases resulting in a frameshift with an impaired protein

rare autosomal dominant disease in humans: Li–Fraumeni syndrome. An impaired p53 pathway as the underlying genetic mechanism of this familial disorder was established in 1990 [11] when Srivastava et al. observed the lost integrity of TP53 in two affected families. While TP53 mutations in Li–Fraumeni syndrome are inherited germline mutations, somatic mutations in cancer cells are most likely induced by replicative and oxidative stress, hyperproliferative signals, and nutrient deprivation. Furthermore, inactivation of p53 can be induced via an imbalance of p53 and its antagonist MDM2 via inactivation of ATM or ATR or through inappropriate activation of the Ras protein. Meanwhile a variety of biological processes have been linked to TP53 inactivation [12]. The most important affect DNA repair and genomic stability, apoptosis and cell cycle arrest, angiogenesis, and migration including metastasis. Interestingly different levels of p53 have been shown to correlate with different transcriptional activation of specific genes and therefore specific functions [13]. The same is true for TP53 mutations. For example, the E177R mutation in the cooperative binding DNA domain of TP53 removes the apoptotic function of the gene, but does not affect cell cycle control, senescence, and metabolic functions [14]. Lately an oncogenic role of mutated TP53 is being discussed again, as the presence of a potentially functional wildtype allele in the case of heterozygous deletion/mutation fails to be beneficial and as the observed mechanisms of mutated TP53 exceed the simple loss of function effects [15].

7.3 TP53 in CLL

TP53 mutations and/or 17p deletions have been identified in the majority of human cancers [16]. Colorectal cancers and carcinomas of head, neck, and esophagus show TP53 lesions in up to 40% of cases. Hematopoietic malignancies are also affected. In CLL the incidence of mutated or deleted TP53 is <3% of cases in Binet A stage or the pre-malignant MBL state, but increases to 12% at the time of first treatment and to more than 37% in fludarabine refractory cohorts [2, 17, 18]. Mutated or deleted TP53 fulfills all the properties of a driver event. Whole exome sequencing studies have identified more than 50 different drivers in CLL, including drivers both of early events, associated with the development of the malignant disorder, and also of late events, transforming CLL into a more aggressive and therapy-resistant disease. There are several reasons to characterize

deletion of 17p and mutation of TP53 as late accelerating events. First the incidence in early disease stages is very low, especially in comparison to del13q or +12q, which affect together more than 60% of early CLL cases. While del13q and +12q are typically clonal aberrations, present in the majority of a patient's CLL cells, del17q only affects smaller subclones, with a rising fraction at relapse. Lineage analyses, in patients with coexistent aberrations or mutations, designed to identify the sequence of their accruement, show TP53 as typically subclonal and therefore subsequent to the clonal event [19]. Considering these observations, and the fact that TP53 is of prognostic value in several cancers, it is unlikely that it plays a role in the early development of lymphoid diseases. Charting the temporal sequence of TP53 mutations and del17p in patients assessed at several time points reveals that both abnormalities occur simultaneously and increase after therapy. This is not self-evident, as conversely it was recently shown that deletion of 11q precedes ATM mutations in the same patient [19]. These observations are consistent with a recent landmark analysis that challenged the applicability of Knudson's classical two-hit hypothesis [20] to sporadic cancer, especially when considering that nearly all cases with deletion of 17p are affected by a TP53 mutation of the other allele, but only 50–60% of TP53 mutated cases have a 17p deletion. For about half of the cases only one of Knudson's two hits was sufficient to adversely affect outcome. However, as patients with mutated TP53 were shown to have several different mutations within the same gene, it is possible that the wildtype allele may also be inactivated by a mutation, or by another mechanism such as expression modification or hypermethylation, rather than by a deletion.

7.4 The Association of TP53 Abnormalities with Other Genetic and Clinical Features

Although TP53 mutation/deletion status is one of the most prominent prognostic factors in CLL, many others have been identified including clinical, biological, chemical, and genetic features. The most informative analyses of the associations between different disease characteristics derive from prospective clinical multicenter trials, such as the CLL1 or CLL8 trial of the German CLL Study Group (GCLLSG). However, as each trial is restricted in regards to Binet stage, age, or number of prior therapies, analyses of large patient cohorts with mixed characteristics are also useful.

Interestingly, mutated TP53 is not associated with lymphadenopathy, splenomegaly, or bone marrow infiltration nor with any other clinical or laboratory feature of the disease at the time of first treatment initiation [2]. Regarding genetic characteristics, abnormal TP53 is associated with unmutated IGHV with only 20% of TP53 mutated patients or 17p deleted patients having mutated IGHV, in contrast to 40% of wildtype patients. When evaluating the association with other chromosomal aberrations in patients with a TP53 mutation, we find a lower incidence of del13q and +12q, while +11q is rare [2, 21]. There is evidence for a synesthetic lethality when both ATM and TP53 pathways are impaired and therefore such a co-incidence may be unfavorable for the tumor clone, especially in the context of chemotherapy resistance [22].

7.5 TP53 in the Context of Clinical Trials and Chemotherapy

TP53 is only one of many recurrently mutated genes identified in CLL. Although mutations in other genes like SF3B1 or NOTCH1 are more common, and some like BIRC3 or XPO1 have relevance for therapy resistance and Richter transformation (see Chap. 4), TP53 is the most important and therefore also the best characterized gene recurrently mutated in CLL. Mutated/deleted TP53 is the only genetic abnormality defining ultra-high risk CLL. Patients with del17p have a median treatment-free interval of only 9 months in comparison to more than 90 months in patients with 13q as the only recurrent genetic abnormality [1]. The association of del17p with refractoriness to purine analogs was

first described 22 years ago and has been validated in many clinical trials since then [23]. The CLL8 trial of the GCLLSG assessed the genetic factors of CLL in younger patients immediately prior to first-line therapy. At this stage 8.2% of patients were positive for del17p and received the standard therapy of fludarabine plus cyclophosphamide (FC) or FC plus rituximab (FCR). After FCR only 5% of 17p deleted patients achieved a complete remission (CR), while the CR rate of wildtype patients was 46%. Three years after receiving FCR only 18% of patients with del17p were without disease progression, compared to 65% of wildtype patients [24]. MRD levels were also significantly higher in patients with del17p [25]. Del17p was not only associated with shorter PFS and OS but also with other adverse prognostic factors within the CLL8 trial. A multivariable analysis of the effect of various prognostic variables on PFS and OS was performed to determine their independent prognostic value, not including gene mutation analysis. Del17p was the strongest prognostic factor in the trial with a hazard ratio of 7.49 for PFS and 9.32 for OS [24]—a result representative of many other previously published studies. This impact is not restricted to purine analogs but has been confirmed for bendamustine, chlorambucil, and other treatment regimens [26, 27]. When the mutation status of TP53 and other recurrently mutated genes was assessed in the CLL8 trial, 11.5% of patients were found to be TP53 mutated prior to first-line therapy [2]. In six of ten TP53 mutated patients a del17p was present. Therefore, it is not surprising to observe an association between TP53 mutation and MRD positivity, lower overall response to therapy, and significantly shorter PFS and OS (Fig. 7.1c and d). However, a multivariate analysis which included TP53 mutation status identified the impact of TP53 mutation to be independent of del17p for both PFS and OS. Interestingly the hazard ratio of del17p and TP53 mutation was similar [2]. Therefore, patients with a TP53 mutation suffer shorter survival irrespective of their del17p status, increasing by 30–50% the number of patients correctly assigned to CLL with high-risk genetics [28, 29]. TP53 mutation and 17p deletion are considered of equal status when it comes to therapy recommendations or assessment of prognosis, including within the CLL-IPI scoring system, which is one of the best tools for CLL prognostication. Therefore, it is very important to assess TP53 status via gene sequencing or following techniques in addition to FISH—a recommendation still not implemented in routine clinical practice in many centers. There are several reasons why treatment with (immuno)chemotherapy is not recommended for patients with diminished p53 activity. From the biological standpoint, malfunctions in DNA repair can lead to an increase in genomic instability within tumor cells, especially when using DNA damaging agents and such instability is linked to more aggressive disease [30, 31]. Regarding clonal evolution, the subclone with defective p53 expands disproportionately at the time of relapse, due to greater resilience or a selective advantage, which can again result in more aggressive disease. From the clinical standpoint, these patients benefit most from novel compounds in terms of response to therapy and PFS.

7.6 TP53 in the Context of Novel Compounds

Even before the era of BCR or BCL2 inhibitors patients with TP53 mutation and del17p were allocated to a chemotherapy-free regimen with alemtuzumab, as usage of this CD52 antibody resulted in similar efficacy independently of TP53 status [32–34]. Currently, with the approval of BCR and BCL2 inhibitors, new drugs with superior outcome and improved safety profile are available. In 2014 the FDA and EMA approved the BTK inhibitor ibrutinib and the PI3K inhibitor idelalisib for relapsed and refractory patients as well as for untreated patients with TP53 mutation or 17p deletion, based on the results of the Resonate Trial [35]. About a third of the patients within the trial had a 17p deletion, and half were TP53 mutated. At 24 months ibrutinib was shown to be substantially superior to ofatumumab with a PFS rate of 74% versus 8% and this difference was seen in all genetic subgroups. While mutation status of IGHV, mutations in

NOTCH1, SF3B1, and BIRC3, and del11q were not associated with inferior outcome in the ibrutinib arm, still TP53 mutated and 17p deleted patients showed a shorter PFS [35]. This prognostic impact is also confirmed in an update of a phase Ib/II trial with ibrutinib as a single agent. After a follow-up of 30 months patients with del17p had a PFS rate of 60% vs. 85% and an OS rate of 80% vs. 90% in comparison to patients lacking del17p and del11q (Fig. 7.3) [36]. Although del17p status impairs efficacy of novel agents, the outcome of this high-risk subgroup was much better than in any historical study with chemotherapy. Therefore, trials exclusively for patients with 17p deletion or TP53 mutation were initiated. A phase 2 trial with 145 pretreated, 17p deleted patients receiving Ibrutinib as a single agent showed remarkable overall response rate of 83% after the median follow-up time of 27.6 months [37]. The estimated PFS and OS at 24 months were 63% and 75%, respectively, and 17 of the 39 patients with progressive disease had a Richter transformation. Even more promising results derived from a cohort of 33 treatment naïve patients with del17p/TP53 mutation: 32 showed a response to ibrutinib as a single agent

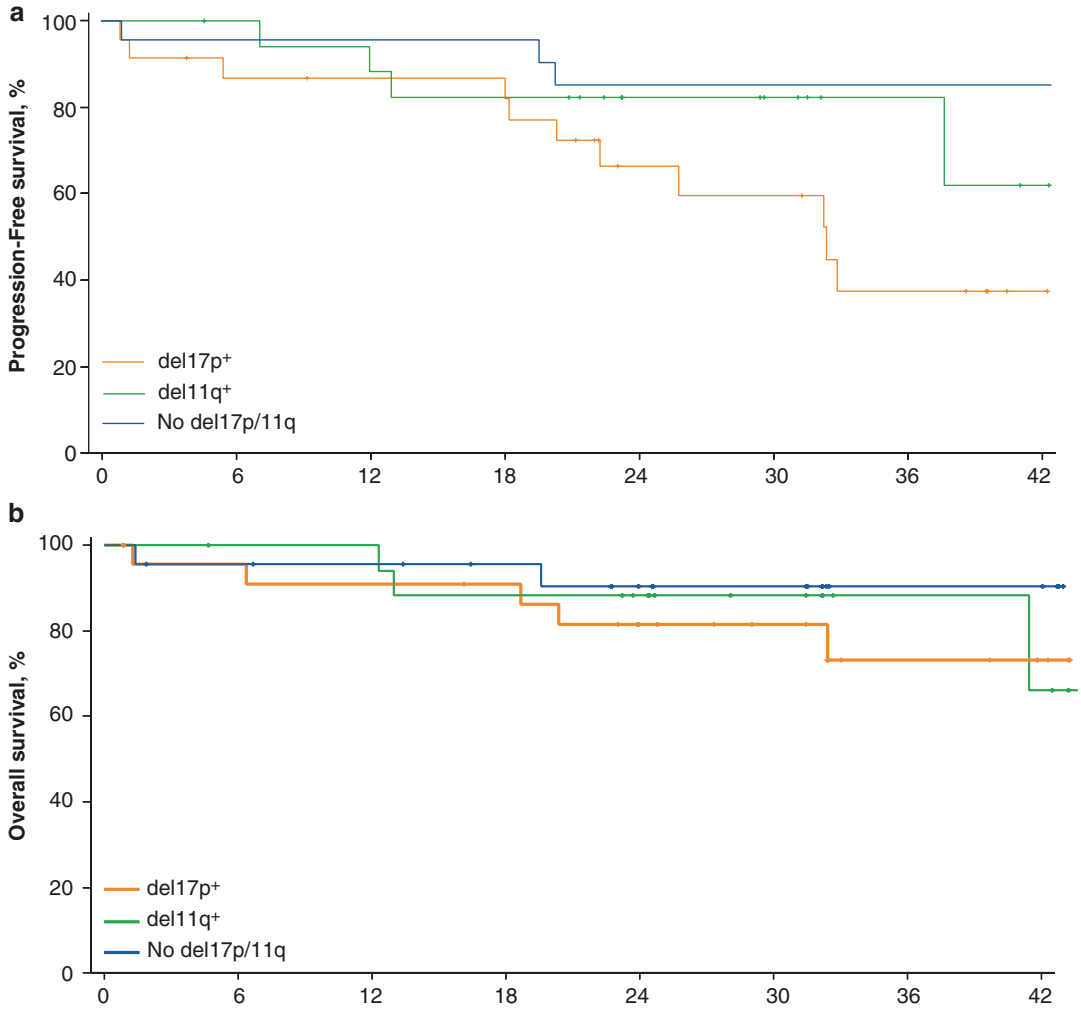

Fig. 7.3 Prognostic value of del17p in the context of novel compounds: outcomes by cytogenetics (FISH) in relapsed refractory (R/R) patients with CLL and Ibrutinib therapy. Progression-free survival (**a**) and overall survival (**b**) by cytogenetic subgroup [36]

after 24 weeks [38]. Meanwhile other trials confirmed the superiority of ibrutinib against other widely used compounds in CLL like chlorambucil or monoclonal antibodies [37, 39, 40]. Although, the prognostic value still remains present in the context of BCR inhibitors, a majority of high-risk CLL patients showed a durable response not seen before in any trial with relapsed/refractory patients and therefore this group of patients may benefit most from this novel therapy.

There is new evidence that multiple chromosomal aberrations are associated with ibrutinib resistance, similarly to del17p, with a high number of cases showing both deletion and complex karyotype. Furthermore, in multivariate analysis complex karyotype competes with del17p as one of the strongest predictors for treatment resistance [38, 41]. However, there are still technical issues with chromosomal banding analysis especially regarding time-critical events like treatment initiation. Also, the assessment of complex karyotype was not included in protocols of some studies and therefore there is still a lack of data from prospective trials.

Regarding the PI3K inhibitor idelalisib, the prognostic value of del17p/TP53mut is less clear. Trials have shown superiority of idelalisib against rituximab monotherapy across all subgroups including high-risk CLL patients. Notably del17p/TP53mutation had a reduced PFS with rituximab as single agent but not with idelalisib, albeit with a relatively short follow-up time [42]. Updated follow-up analysis is not yet available and long-term results from other clinical trials are missing as several trials were stopped due to grade 4 and 5 side effects of idelalisib, also observed in previously untreated patients. This resulted in a withdrawal of the approval of idelalisib as a first-line regimen for high-risk CLL cases. In summary the BCR-inhibitors ibrutinib and idelalisib are both very efficacious in disease control, but fail to achieve MRD negativity in most patients, especially within the first year of treatment.

In contrast the BCL2 inhibitor venetoclax results in a quick reduction of the tumor load and deep remissions. The M13-982 trial for pretreated high-risk CLL patients confirmed its efficacy in del17p patients [43] by achieving an ORR of almost 80%. A comparable rate was achieved in the phase I trial open for all relapsed/refractory cases [44]. A high percentage of MRD negative cases, translating into durable remissions, was not achieved in comparable study cohorts in the past. However, outcome data within different subgroups, and also the value of prognostic factors established in the era of chemotherapy, are under investigation in current trials. Combination therapies of novel antibodies and novel compounds are being tested in phase 2 trials, but studies comparing two or three different drugs are rare and results are not yet available. Although a therapy sequence for high-risk CLL patients is not yet established, there are several factors to be considered in daily clinical practice including concomitant diseases, possible drug interactions, toxicities, and adherence to prior therapy lines, all of which will guide the physician in choosing the most suitable compound. Additionally, there are approval restrictions for venetoclax and idelalisib. However, as long as novel compounds fail to cure CLL, one major question remains crucial for the choice of therapy for high-risk CLL patients: their eligibility for an allogeneic stem cell transplant.

7.7 Allogeneic Stem Cell Transplantation Remains the Treatment with Curative Potential

The role and optimal time point for an allogeneic stem cell transplant (SCT) in patients with del17p and TP53mut is unclear. This position has not changed with the advent of ibrutinib, idelalisib, and venetoclax. For several years younger patients with high-risk CLL, defined by cytogenetics and/or early relapse, were advised to consider allogeneic SCT. This treatment has been shown to achieve long-term remissions, as demonstrated in a 10-year follow-up of the GCLLSG CLL3X trial. In total 90 transplanted high-risk CLL patients, 35% with deletion of 17p/TP53 mutation, underwent a fludarabine based reduced intensity conditioning regimen followed by allogeneic transplantation from a matched related or

unrelated donor. The PFS and OS rates were, respectively, 34% and 51%, irrespective of 17p/TP53 status. Thirty-two patients achieved a long-term remission after therapy, while another 39 patients relapsed mainly in the early follow-up period. It is noteworthy that 20 of 90 patients died of CLL and another 17 of non-relapse mortality. Efficacy data from the European Society for Blood and Marrow Transplantation are available from 694 CLL patients after allogeneic transplantation. The OS rates in this multicenter register were 64% and 47% after 2 and 5 years, respectively [45]. Non-relapse mortality at 2 and 5 years was higher than the incidence of relapse/progression and emphasizes the enhanced risks of SCT in comparison to any other type of therapy. The independent risk factors for non-relapse mortality, identified via multivariate analysis, were higher age, reduced performance status, HLA disparity, and unfavorable donor–recipient sex match. Presence versus absence of all these risk factors together resulted in 42% vs. 11% non-relapse mortality. Hence, consideration of these risk factors is crucial for therapy guidance of CLL patients eligible for allogeneic HCT. Although the early-death rate (<100 days after transplant) has decreased below 5% with modern transplant strategies and avoidance of T-cell depletion, non-relapse mortality still amounts to 15–30% of all patients, mainly due to GvHD related complications [46]. In addition, death due to progression occurred in 15–25% of patients. A previous autologous transplant, and particularly remission status at conditioning, were identified as independent prognostic factors for relapse [45]. Although no prospective trials of transplant versus conventional therapy are available, the adverse outcome in patients not receiving a transplant due to absence of a matching donor underlined the importance of SCT [47] in the era of chemotherapy. In this trial patients had no access to novel agents and therefore today such a study may have different results. The greater efficacy of novel compounds, used both as first-line and salvage treatment in 17p deleted/TP53 mutated patients, may change the course of the disease and the patients' prognosis. In some cases it may also delay allogeneic SCT to a time point at which the patient's age may make an allogeneic transplant less favorable. However, the last 10 years of drug development in CLL give confidence that another generation of novel agents may emerge in the near future suitable for patients who are resistant to BCR- and BCL2-inhibitors.

In conclusion, and following the 2018 ERIC- and EBMT-guidelines [48], patients with del17p/TP53 mutation who have relapsed after one or more rounds of immunochemotherapy, but who are naïve for novel compounds, should be considered for allogeneic SCT and informed about the morbidity and mortality risks described above. Patients will typically start with ibrutinib or venetoclax to achieve remission before allogeneic transplant, which will allow the opportunity to include factors such as treatment tolerability and MRD negativity in the subsequent decision making. For patients who have relapsed after both chemoimmunotherapy and at least one novel compound, rescue options are limited. Therefore, in this subgroup an allogeneic transplantation should be given more favorable consideration. BTK- and BCL2 inhibitors can optimize the outcome of SCT due to a deeper remission prior to conditioning and can also be used as an efficacious therapy at relapse after SCT [49]. Hence, in a majority of high-risk CLL patients novel compounds and SCT are complementary rather than competing treatment options.

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8 Treatment of Relapsed and Refractory Chronic Lymphocytic Leukemia

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8.1 Introduction

Despite the recent advances in CLL treatment and the introduction of novel, more effective drugs, the disease remains incurable in relapse situation with the exception of allogeneic hematopoietic stem cell transplantation (alloHCT). Most patients will eventually relapse, and some are refractory to treatment. According to the International Workshop Group on CLL (IWCLL) guidelines, relapsed patients are defined as those who have previously achieved a complete response (CR) or partial response (PR) but demonstrated evidence of disease progression after a period of six or more months [1]. Treatment-refractory CLL is defined as a disease without PR or CR, or disease progression within 6 months following the last antileukemic therapy. The response to second or subsequent line treatment depends on a variety of factors including clinical stage, adverse biological prognostic factors, and numbers of prior therapies, particularly those with purine analogs. Patients refractory to previous therapy, especially with fludarabine-containing regimens, and those with a del17p/*TP53* mutation have particularly poor prognosis [2]. In studies, del17p has been identified in around 7% of previously untreated patients and as many as 50% with relapsed/refractory disease. Disease progression within 2 years of the initiation of frontline therapy is an independent negative predictor of survival [3].

Treatment decisions in relapsed patients follow the same criteria as those used for the initiation of first-line treatment [1]. Therapy for relapsed and refractory patients should be planed according to the clinical stage of disease, fitness of the patients, and response to the previous treatment, as well as laboratory parameters such as renal function, bone marrow reserve, and cytogenetics. Repeat FISH testing and molecular testing for *TP53* mutation at the time of relapse is important to optimize treatment for high-risk patients.

The recently approved B-cell receptor (BCR) signaling inhibitors, ibrutinib and idelalisib, as well as the B-cell lymphoma 2 (BCL-2) inhibitor venetoclax, and the novel antibodies, obinutuzumab and ofatumumab, have significantly improved the outcomes of relapsed and refractory CLL patients [4–8].

8.2 Management with Late Relapsed Patients

Patients who relapse after long-lasting remission and have not acquired a *TP53* abnormality can be expected to respond to a further course of their initial therapy, although progression-free survival (PFS) is usually shorter than after initial therapy

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and repeated courses often lead to drug resistance [9]. However, retreatment is not indicated in patients with sub-optimal previous therapy or if more effective therapy becomes available. Recent European Society for Medical Oncology (ESMO) recommendations indicate that first-line treatment may be repeated if the relapse or progression occurred within 24–36 months or longer after initial therapy [9]. However, in the era of new drugs, only a small proportion of relapsed patients are retreated with previous therapies.

An analysis of the effect of subsequent therapies in patients with CLL from five prospective phase II/III trials conducted between 1999 and 2010 by the German CLL Study Group (GCLLSG) found that the same therapeutic regimen was repeated in a subsequent treatment line in only 122 of 704 (17.3%) relapsed patients [10]. Moreover, only 55 of 368 patients (14.9%) who started second-line treatment more than 24 months after first-line therapy received the first-line regimen again in the second line, and 43 patients received repeated treatment with fludarabine or fludarabine and cyclophosphamide (FC) in the second line. The median event-free survival (EFS) was only 5.7 months in patients receiving the same purine analog chemotherapy within 24 months, compared to 18.2 months in patients repeating the treatment after 24 months ($p = 0.071$), with the respective median overall survival (OS) values for the two groups being 26.6 and 82.3 months ($p = 0.6$). The authors suggest that a threshold of 24 months is an appropriate time for a repetition of purine analog-based chemotherapy and 36 months for chemoimmunotherapy.

The PALG (Polish Adult Leukemia Study Group) study found retreatment with cladribine (2-CdA) leads to response in about half of the patients [11]. However, the duration of response was shorter in retreatment than after first-line treatment and myelotoxicity was more pronounced. In an analysis performed by the French intergroup group, a cut-off of 36 months was found to properly differentiate patients for retreatment with FCR (fludarabine, cyclophosphamide, rituximab) because patients with a relapse within 36 months after FCR demonstrated poor results similar to those of patients with relapses before 24 months [12]. Therefore, retreatment with aggressive chemotherapy or immunochemotherapy is not recommended in patients with an early relapse. In addition, FCR is associated with significant toxicity, including grade III–IV neutropenia and severe infections, accumulated myelotoxicity, and second neoplasms [13].

Retreatment with bendamustine, either used alone or in combination with another agent, is also effective in CLL patients. Welde et al. examined 57 patients with CLL previously treated with bendamustine and then retreated with either a combination of bendamustine and rituximab or of bendamustine, mitoxantrone, and rituximab [14]. The overall response rate was 77% including 6% CR. Bendamustine retreatment is feasible and achieves high response rates and some long-lasting remissions.

8.3 Treatment of Relapsed and Refractory Patients with Conventional Drugs

The results of randomized studies suggest that, in patients with relapsed/refractory CLL, the combination of the anti-CD20 monoclonal antibodies (mAb) rituximab or ofatumumab with fludarabine and cyclophosphamide (FC) can improve the treatment outcome to a greater degree than the use of FC alone. A multicenter, randomized phase III trial (REACH) compared six cycles of FCR (FC + rituximab) with six of FC in previously treated patients with refractory or relapsed disease (Table 8.1) [15]. The majority of the patients were pretreated with alkylators, mainly chlorambucil; however, one fourth of the patients were refractory to these agents. After a median follow-up time of 25 months, PFS was significantly higher in the FCR group (median value 30.6 months) than the FC group (median value 20.6 months) ($p < 0.001$). The overall response (OR) rate and PFS were also better in the FCR arm (Table 8.1), and the CR rate was significantly higher in the FCR arm (24.3%) than the FC arm (13.0%). In addition, patients receiving FCR

Table 8.1 Randomized clinical trials in relapsed/refractory CLL

Study	Treatment	Number of patients	Median age	Previous regimens	Overall response rate	Complete response rate	Progression-free survival	Overall survival
REACH Robak et al. [15]	FCR vs FC	276 vs 276	62 years vs 63 years	Refractory 27% vs 26%	69.9% vs 58.0% $p = 0.0034$	24.3% vs 13.0% $p < 0.001$	30.6 m vs 20.6 m $p < 0.001$	NR vs 52 m $p = 0.2874$
COMPLEMENT 2 Robak et al. [16]	OFC vs FC	183 vs 182	62 years vs 63 years	Median (range) 1 (1–8) vs 1 (1–6)	84% vs 68%	27% vs 7%	28.9 m vs 18.8 m $p = 0.0032$	56.4 m vs 45.6 m $p = 0.1410$
LUCID Awan et al. [17]	Lumiliximab + FCR vs FCR	316 vs 311	61 years vs 61 years	Prior F 52% vs 58%	71% vs 72% $p = 0.92$	16 vs 15 $p = 0.782$	24.6 m vs 23.9 m $p = NS$	Not reached vs not reached $p = NS$
CAM 314 Elter et al. [18]	Alemtuzumab + F vs F	168 vs 167	60.0 years vs 60.8 years	Prior F 15% vs 16%	82% vs 75% $p = 0.18$	13% vs 4% $p = 0.06$	23.7 m vs 16.5 m $p = 0.0003$	Not reached vs 52.9 m $p = 0.021$
RESONATE I Byrd et al. [5]	Ibrutinib vs Ofatumumab	195 vs 196	67 years vs 67 years	Median (range) 3 (1–12) vs 2 (1–13)	42.6% vs. 4.1% $p < 0.001$	2 vs 1	Not reached vs 8.1 m $p < 0.001$	At 12 months 90% vs 81% $p = 0.005$
HELIOS Chanan-Khan et al. [19]	Ibrutinib + BR vs BR	289 vs 289	64 years vs 63 years	Mean (range) 2 (1–11) vs 2 (1–9)	82.7% vs 67.8% $p < 0.0001$	10.4 vs 2.8	NR vs 13.3 m $p < 0.0001$	NR vs NR $p = 0.0598$
Study 116 Furman et al. [6]	Idelalisib + rituximab vs rituximab	110 vs 110	71 years vs 71 years	Median no. of drugs (range) 3 (1–12) vs 3 (1–9)	81% vs 13% $p < 0.001$	0 vs 0	NR vs 5.5 m $p < 0.001$	At 12 m 92% vs 80% $p = 0.02$
Study 119 Jones et al. [20, 21]	Idelalisib + ofatumumab vs ofatumumab	174 vs 87	68 years vs 67 years	Median (range) 3 (2–4) vs 3 (2–5)	75.3% vs 18.4% $p < 0.0001$	<1 vs 0	16.3 m vs 8.0 m $p < 0.0001$	20.9 m vs 19.4 m $p = 0.27$
Study 115 Zelenetz et al. [22, 23]	Idelalisib + BR vs BR	207 vs 209	62 years vs 64 years	Refractory 31% vs 29%	68% vs 45%	5 vs 0	23.1 m vs 11.1 m $p < 0.001$	NR vs NR $p = 0.008$

Abbreviations: *NR* not reached, *O* ofatumumab, *BR* bendamustine + rituximab, *F* fludarabine, *FC* fludarabine + cyclophosphamide, *FCR* FC + rituximab, *OFC* ofatumumab + FC

demonstrated significantly better event-free survival (EFS), duration of response, and time to new CLL treatment or death. However, patients with poor-risk cytogenetics, including abnormalities of chromosome 17p, patients with fludarabine-refractory CLL, or heavily pretreated patients with more than three prior treatments continue to have poor prognosis.

The FCR regimen has also been combined with a fourth agent, such as mitoxantrone, or a second monoclonal antibody like alemtuzumab or lumiliximab, but the results were not significantly different from those obtained with FCR alone [17, 24, 25]. In addition, many patients with relapsed/refractory CLL are unable to tolerate immunochemotherapy based on FCR due to regimen-related toxicity. Moreover, individuals with del17p/*TP53* mutations show very low response rate and short duration remission. Chemoimmunotherapy is also not effective in patients who discontinue BCR inhibitor therapy. A recent study found only 25% of the patients who failed ibrutinib or idelalisib treatment responded to treatment with the combination of mAbs and cytotoxic drugs [26].

Bendamustine has shown efficacy in relapsed/refractory CLL, especially in combination with rituximab (BR), in patients who have received prior therapy with other alkylating agents or purine analogs: A phase II study found it to elicit response rates of 56–60% in heavily pretreated patients [27, 28]. The most common grade 3/4 toxicities were generally hematological: granulocytopenia and thrombocytopenia in particular. Rituximab combined with bendamustine (BR) has been proposed as a less toxic regimen than FCR and is commonly used in relapsed or refractory patients [29]. An evaluation of the efficacy and safety of BR in 78 patients previously treated with fludarabine by Fisher et al. reported an OR rate of 59.0% with 9% CR. Fludarabine-refractory patients responded in 45% of cases and fludarabine-sensitive patients in 60%. However, only 7% of patients who responded to the BR treatment were found to possess del(17p). The most frequent adverse events (AEs) were myelosuppression and infections. Because of its efficacy and their favorable toxicity profile, the combination of bendamustine and rituximab is frequently used in first line and further lines of therapy in patients with CLL.

Ofatumumab is the first fully human anti CD-20 mAb targeting a novel epitope of the CD-20 molecule on B-cells. It has similar antibody-dependent cellular cytotoxicity (ADCC) to rituximab, but it releases very slowly from the target and possesses stronger complement-dependent cytotoxicity (CDC). The results of a large multicenter trial of single-agent ofatumumab in 138 patients with CLL refractory to fludarabine and alemtuzumab (FA) led the approval of ofatumumab for FA-refractory patient populations in the USA and Europe [30, 31]. It found OR rates of 58% in the FA-refractory patients, PFS 5.7 months, and OS 13.7 months. In the fludarabine-refractory patients with bulky lymphadenopathy (BFR) OR was 47%, PFS 5.9 months, and OS 15.4 months. Unfortunately, patients with del17p responded poorly, suggesting limited activity of ofatumumab in this subgroup. Overall, these results suggest that ofatumumab can achieve disease control with symptomatic improvement in a subset of patients, but its impact on survival is limited.

Similarly to FCR, ofatumumab combined with FC (OFC) improved PFS in patients with relapsed CLL compared with FC alone. A multicenter, open-label, phase III study (COMPLEMENT 2) performed in patients with relapsed CLL found median PFS to be 28.9 months with OFC versus 18.8 months with FC (p = 0.0032) with manageable safety for patients with relapsed CLL compared with FC alone (Table 8.1) [16]. The incidence of grade 3 or higher AEs was 74% in the OFC arm and 69% in the FC-only arm. Neutropenia was the most common of these events, occurring in 49% of OFC patients and 36% of FC patients. In 2016, the European Commission granted marketing authorization for ofatumumab to be used in combination with FC in the treatment of patients with relapsed CLL. A phase II, noncomparative study of the efficacy and safety of bendamustine in combination with ofatumumab performed on 47 patients with relapsed/refractory CLL achieved an OR rate of 72%, with 17% CR and median

PFS of 23.6 months [32]. After a median follow-up of 24 months the PFS was 50% and OS 84%. This regimen can be an alternative treatment option for patients with relapsed CLL.

Obinutuzumab (GA-101) is a novel third-generation mAb with higher affinity to the CD20 type II epitope and more enhanced induction of ADCC in comparison with rituximab. Obinutuzumab monotherapy is active in patients with heavily pretreated relapsed/refractory CLL. The phase 1/2 GAUGUIN study demonstrated that obinutuzumab can be safely administered to patients with relapsed/refractory CLL at doses up to 2000 mg [33]. The best OR values were 62% during phase 1 and 30% in phase 2. Phase 2 median PFS was 10.7 months and median duration of response was 8.9 months. However, the antibody is so far only approved in frontline in combination with chlorambucil, but not in relapse therapy and not as monotherapy. Obinutuzumab has potential for combination with new BCR inhibitors such as ibrutinib or idelalisib, and clinical trials with such combinations have been initiated.

Alemtuzumab is a humanized mAb against CD-52, an antigen expressed on B-cells, T-cells, and almost all CLL cells. Alemtuzumab is effective regardless of cytogenetic risk group, including high-risk chromosome 17p-deleted and fludarabine-refractory patients. Single-agent alemtuzumab induces a response in up to 40% of patients with fludarabine-refractory CLL, but responses are not durable, and the median survival is approximately one to 2 years. In a pivotal registration trial by Keating et al., the OR rate was 33% (CR 2%, PR 31%) and the median time to progression was 9.5 months for responders [34]. However, PFS following alemtuzumab monotherapy was short, with a median PFS of only 10–13 months for responders. While the median OS was 16 months for the study population as a whole, this value improved to 32 months for responders alone. In addition, patients with bulky lymphadenopathy generally have poor responses after alemtuzumab monotherapy. Major side effects included infusion reactions associated with IV administration and infections.

Stilgenbauer et al. administered alemtuzumab subcutaneously on an outpatient basis, at 30 mg three times weekly in the CLL2H trial [35]. The overall response rate was 34% (CR 4%), median PFS was 7.7 months, and median OS 19.1 months. Subcutaneous administration was more convenient for the patients. The CLL2H trial also confirmed prior reports that alemtuzumab induces similar responses and outcomes in patients with and without del17p. Among patients with del11q and del17p cytogenetic abnormalities, the response rates were 39% and 24%, respectively. Progression-free survival and OS did not differ significantly among the genetic subgroups, particularly mutated *TP53*, del17p, and del11q. The most common toxicities included hematologic toxicities (grade 3–4 anemia—42%, thrombocytopenia—52%, neutropenia—54%, and infections). These findings indicate that alemtuzumab had activity in patients with high-risk CLL, including those with unmutated *IgVH*, del11q, or del17p.

Alemtuzumab was combined with fludarabine (FluCam) or rituximab with a significant responsiveness and acceptable toxicity. FluCam showed excellent results, with an OR rate of 83% and CR rate of 30% in relapsed or refractory patients, with a time to progression (TTP) of 36 months for all heavily pretreated patients [36]. These results have been confirmed in a large randomized phase III trial (CAM 314), comparing FluCam with fludarabine monotherapy in 335 patients with relapsed or refractory disease (Table 8.1). The CAM 314 trial demonstrated that both PFS and OS were significantly for the FluCam combination than fludarabine alone [18]. However, alemtuzumab is associated with a risk of toxicity, especially bacterial and viral infections; these are usually manageable with standard therapies, and combined antimicrobial prophylaxis and cytomegalovirus monitoring are compulsory. However, this drug is no longer used in relapsed patients as treatment with novel drugs is now available in most countries. Alemtuzumab was withdrawn from the market in 2012, and is not commercially available.

High-dose methylprednisolone (HDMP), either administered alone or in combination with

rituximab, is a palliative treatment in patients with relapsed/refractory CLL, including fludarabine-refractory patients and cases with the *TP53* mutation. Castro et al. found combined rituximab and HDMP therapy to have an OR rate of 93% and a CR rate of 36% when used as a salvage regimen for the treatment of patients with fludarabine-refractory CLL [37]. The median time-to-next treatment was 22 months and the median PFS was 15 months. The Mayo Clinic group reported data on this combination in 37 CLL patients and reported an OR rate of 78%, including 22% CR [38]. In patients with del17p deletion, an OR rate of 55.6% was observed. Durngwala et al. found HDMP plus rituximab to be more effective than historical controls treated with HDMP alone with respect to OR (93% vs. 43%) and CR (14% vs. 0%) [39]. Some concerns over this treatment combination included the high incidence of infections. HDMP and rituximab are non-myelosuppressive agents and therefore are suitable for patients with cytopenias. However, previous significant incidences of infections have been observed and *Pneumocystis* pneumonia (PCP) prophylaxis is recommended.

8.4 Inhibitors of the B-Cell Receptor Pathway

A better understanding of the mechanisms underlying CLL has implicated BCR activation in its pathogenesis [40, 41]. Ibrutinib, a first-in-class Bruton's tyrosine kinase (BTK) inhibitor, and idelalisib, an inhibitor of the delta isoform of phosphoinositol-3 kinase (PI3Kδ), are both first-in-class agents that target the BCR cascade [6, 42]. These drugs address an unmet need by providing treatment options with tolerable safety profiles without compromising survival in the second-line setting. Both drugs were approved in 2014 for CLL patients with relapsed/refractory disease [43]. BCR inhibitors offer durable remission coupled with modest toxicity in relapsed/refractory CLL. Since their approval, BCR inhibitors have changed the treatment landscape and horizon for patients with relapsed/refractory disease.

8.4.1 Ibrutinib

Ibrutinib forms a covalent bond with a cysteine residue in the BTK active site, leading to inhibition of enzymatic activity. This drug demonstrated encouraging results in a pivotal, phase 1b–2 multicenter study performed in 48 previously treated participants who had received four previous therapies [5]. The patients received ibrutinib 420 mg/day until unacceptable toxicity or disease progression. The OR rate was nearly 58% and the duration of response ranged from 5.6 to 24.2 months. Ibrutinib also represents a clinical advance in the treatment of relapsed or refractory patients with del17p. A study of 144 patients with del17p CLL who had received a median of two previous treatments found an OR rate of 83% and 24-month PFS of 63% following ibrutinib treatment [44].

Ibrutinib was compared with ofatumumab in a large, multicenter, phase 3 study (RESONATE I), performed in 391 patients with relapsed or refractory CLL (Table 8.1) [5]. The OR rate was significantly higher in the ibrutinib group than in the ofatumumab group (42.6% vs. 4.1%, $p < 0.001$). The median duration of PFS was not reached in the ibrutinib group, while median PFS was 8.1 months in the ofatumumab group ($p < 0.001$). Ibrutinib administration also significantly improved the OR and the OS rates. The OR at 12 months was 90% in the ibrutinib arm and 81% in the ofatumumab arm. In February 2014, the FDA approved ibrutinib for CLL in patients who had received at least one previous therapy. Subsequently, the FDA approved an expanded indication for ibrutinib for the treatment of CLL patients with a deletion in chromosome 17.

In recent trials, ibrutinib was combined with other drugs, including rituximab and bendamustine [45, 46]. Ibrutinib combined with rituximab only was investigated in a single-arm, phase 2 study in 40 patients with high-risk CLL, and the results seem to be similar for those obtained with ibrutinib plus BR [46]. A recent randomized phase 3 study (HELIOS) compared ibrutinib plus BR with BR alone in patients with previously treated CLL (Table 8.1) [19]. The results demonstrated that the combination of ibrutinib with BR

is a more effective treatment for relapsed CLL patients with high-risk disease than BR alone. Progression-free survival was significantly longer in the ibrutinib group (median not reached) than in BR alone (13.3 months) ($p < 0.0001$), and PFS at 18 months was 79% vs 24% ($p < 0.0001$). The safety profile was similar to that previously reported with ibrutinib and BR. These results demonstrate that the addition of ibrutinib to a standard BR regimen results in significant improvements in outcome when compared with standard BR chemoimmunotherapy. It is not known, however, whether the combination of ibrutinib with rituximab, or with BR, is more effective than ibrutinib alone [47].

Preliminary results indicate that patients with CLL sensitive to ibrutinib at the time of alloHCT might benefit from ibrutinib bridging [48]. In the study reported by Dreger et al. 28 patients with CLL were treated with ibrutinib before transplant for a median of 190 (39–432) days [48]. Ibrutinib-sensitive CLL tended to be associated with a lower 1-year relapse (29%) compared to refractory disease status at alloHCT (60%; $p = 0.071$). In contrast *TP53* status, duration of ibrutinib treatment, interval between ibrutinib withdrawal and alloHCT, and conditioning intensity had no significant impact on incidence of relapse. Moreover, ibrutinib does not adversely affect engraftment and graft-versus-host disease (GVHD) risk.

Ibrutinib can also be used after CLL relapse following allogeneic hematopoietic stem cell transplantation (allo-HCT) [49, 50]. In a recent study of 27 patients with relapsed CLL following allo-HCT who subsequently received ibrutinib salvage therapy, an 87.5% OR rate was observed with only three progressions after the 24-month observation [50].

8.4.2 Idelalisib

Idelalisib, an inhibitor of PI3Kδ, demonstrated potent inhibition of BCR signaling, which induces apoptosis and inhibits proliferation of B-cells. A phase 1 trial of idelalisib including 54 heavily pretreated relapsed/refractory CLL patients with a median of five prior regimens returned an OR of 72% with at least nodal responses observed in 81% of patients. The drug was well tolerated with grade 3 or higher pneumonia in 20%, neutropenic fever 11%, and diarrhea in 6% of the patients [51]. In July 2014, the FDA approved idelalisib for the treatment of relapsed CLL in combination with rituximab. Approval was based on a placebo-controlled study of 220 patients, in which those treated with idelalisib plus rituximab showed significantly longer PFS (10.7 months) than those who received placebo plus rituximab (5.5 months) ($p > 0.001$) (Table 8.1) [6]. The OR rate for the combination of idelalisib and rituximab was 81% while that for rituximab alone was 13% ($p > 0.001$). Overall survival was also longer for the idelalisib arm than for the rituximab arm: At 12 months, survival was 92% (idelalisib + rituximab) vs 80% (rituximab) ($p = 0.02$). Idelalisib has also been investigated in combination with ofatumumab, as well as with bendamustine and rituximab [20–23]. The combination of idelalisib with ofatumumab resulted in significant improvements in PFS and response rates compared with ofatumumab alone (Table 8.1) [20, 21]. The median PFS was 16.3 months in the idelalisib/ofatumumab group and 8.0 months in the ofatumumab group ($p < 0.0001$). The combination of idelalisib with ofatumumab was also more effective in patients with high-risk genetic characteristics such as *TP53* disruption and del17p, or *TP53* mutations and unmutated *IGHV* [20, 21]. The addition of idelalisib to ofatumumab was generally well tolerated. The most frequent grade 3 or higher adverse events in the idelalisib plus ofatumumab group were neutropenia (34% vs 16%) and diarrhea (20% vs 1%). Moreover, an increased risk of serious infections was observed in the idelalisib plus ofatumumab group including pneumonia (13% vs 10%), sepsis (6% vs 1%), and *Pneumocystis jirovecii* pneumonia (5% vs 1%).

In another large randomized trial, idelalisib combined with BR was also superior to placebo with BR in improving PFS and OS (Table 8.1) [20, 21]. Median PFS was 23 months in the idelalisib arm and 11 months in the placebo arm ($p < 0.001$) at a median follow-up of 12 months. Median OS was 41 months in the BR only arm and not reached

in the idelalisib arm ($p = 0.036$). Infections were more common in the idelalisib arm (41%) than the placebo arm (23%). Febrile neutropenia was observed in 21% (idelalisib) vs 5% (placebo) and pneumonia in 17% (idelalisib) vs 8% (placebo). According to recent recommendations, patients treated with idelalisib-containing regimens should receive implementation with adequate *Pneumocystis jirovecii* pneumonia prophylaxis and cytomegalovirus (CMV) monitoring measures.

8.4.3 Management with the Patients After Discontinuation of BCR Inhibitors

The most common reasons for discontinuation of BCR inhibitors are toxicity, CLL progression, and Richter syndrome [52, 53]. Mato et al. analyzed the reasons for ibrutinib (143 patients) or idelalisib (35 patients) discontinuation in 187 heavily pretreated patients who had undergone a median of three prior therapies [26]. BCR inhibitor toxicity was the reason for treatment discontinuation in 51% of the patients and CLL progression in 29%. An alternate BCR inhibitor was the most common treatment following BCR inhibitor discontinuation (39%, 44/114). Patients who discontinue BCR inhibitors have a poor prognosis. A recent analysis found alternate BCR inhibitor therapy following initial BCR inhibitor discontinuation to be effective in only 50% of patients. The shortest median PFS after discontinuation had patients with Richter transformation (6 months), followed by those who discontinued BCR inhibitors due to CLL progression (8 months), and BCR inhibitor intolerance (10 months) [26]. Currently, the best option for the treatment of CLL patients who fail ibrutinib or idelalisib therapy is the BCL-2 antagonist venetoclax (see below).

8.5 BCL-2 Antagonists

Venetoclax is a selective inhibitor of the BCL-2 anti-apoptotic protein highly expressed in CLL cells [8, 54]. Potential of venetoclax to yield high responses in patients with relapsed or refractory CLL is continually being validated. When used as a single agent, venetoclax induces objective response in approximately 80% of patients with relapsed/refractory CLL including del(17p), 16–20% of whom demonstrate CR [8]. The M13-982 phase 2 trial reported an OR rate with venetoclax monotherapy of 79.4%, with CR occurring in 7.5% of patients. Similarly to other treatments in hematologic oncology, the most frequently observed Grade 3–4 adverse reaction was neutropenia, occurring in 43% of subjects [7]. In May 2016, venetoclax was approved by the FDA for patients with del17p who had been treated with at least one prior therapy. In October 2016, venetoclax was awarded conditional approval from the European Medicines Agency (EMA) for CLL patients with either del17p or *TP53* mutation and have either failed chemoimmunotherapy or are unsuitable for treatment with a BCR pathway inhibitor such as ibrutinib or idelalisib. Venetoclax demonstrates high activity and good tolerability in patients with CLL refractory to, or progressing during or after treatment with ibrutinib or idelalisib.

Currently, venetoclax seems to be the treatment of choice for the patients who discontinued BCR inhibitor therapy. A recent analysis found 76% OR and 7% CR in CLL patients treated with venetoclax after ibrutinib or idelalisib discontinuation [26, 55]. Elsewhere, a study of 64 patients previously treated with ibrutinib (41) or idelalisib (21) were then treated with venetoclax for a median period of 13 months (ibrutinib) or 9 months (idelalisib). The objective response rate was found to be 30/43 (70%) among those previously treated with ibrutinib and 10/21 (48%) those with idelalisib. In addition, 42 (33%) patients achieved minimal residual disease (MRD)-negativity in peripheral blood between weeks 24 and 48. For all patients, estimated 12-month PFS was 72% and OS 90%. The safety profile of venetoclax remains acceptable, with the most common toxicities being hematologic and gastrointestinal [56]. The combination of venetoclax with rituximab has also been found to be effective and safe in relapsed and refractory CLL patients. A recent study found the OR rate to

be 86% in a study group comprising 49 patients, with approximately half achieving CR [57]. MRD-negativity in bone marrow was noted in 13/20 (65%) patients with CR.

A recent large-scale analysis has provided guidelines for the sequencing of novel agents in relapsed/refractory patients with CLL, based on which venetoclax should be used upon BCR inhibitor failure [58]. Treatment with venetoclax was associated with longer PFS than chemoimmunotherapy combinations in the patients who discontinued BCR inhibitors. However, there is a need for trials directly comparing novel agents and sequencing strategies in this disease.

8.6 Investigational Drugs

Recently, several new agents have shown promise in treating CLL, and the second-generation BTK inhibitors, acalabrutinib (ACP-196) and ONO-4059 (GS-4059 respectively) are now under investigation [59, 60]. Second-generation PI3Kδ inhibitors that are in development to address the safety concerns observed with idelalisib by reducing the severity of associated transaminase elevations [61, 62]. Other PI3Kδ inhibitors under investigation include acalisib (GS-9820), TGR-1202, and duvelisib (IPI-145) [62–64]. Trials are underway for the use of these agents in relapsed/refractory CLL.

The novel anti-CD20 mAb ublituximab (TG-1101) is effective in relapsed/refractory CLL, particularly when combined with ibrutinib [65]. A phase 2 study evaluating combined therapy with ublituximab and ibrutinib revealed rapid and high response rates in patients with relapsed or refractory CLL. An OR rate of 88% was achieved at 6 months in the total population, and this rate grew to 95% including 15% MRD-negativity in 20 patients with 17p or 11q deletions or *TP53* mutation.

Otlertuzumab (TRU-016) is a humanized anti-CD37 protein therapeutic that induces ADCC and triggers direct caspase-independent apoptosis of malignant B-cells. A recent randomized study compared bendamustine plus otlertuzumab therapy with the use of bendamustine alone in patients with relapsed CLL [66]. The combination significantly increased the response rate and prolonged the PFS over single-agent bendamustine in patients with relapsed or refractory CLL. Overall response rate was 69% in the otlertuzumab and bendamustine arm and 39% in the bendamustine alone arm (p = 0.025). Median PFS was also longer in the otlertuzumab combination arm (15.9 months) than in bendamustine alone (10.2 months) (p = 0.0192). However, it is not clear whether this anti-CD37 agent is more effective in CLL patients than the anti-CD20 antibodies.

PD-1 blocking antibodies are also active in CLL. In the MC1485 trial, the PD-1 blocking antibody pembrolizumab was administered intravenously at a dose of 200 mg every 3 weeks in patients with relapsed/refractory CLL, including those with Richter syndrome [67]. The OR rate was found to be 16% across this group of heavily pretreated patients, rising to 44% for those with Richter syndrome. The combination of nivolumab with ibrutinib is the topic of another ongoing study, but only preliminary results are currently available [68].

8.7 Allogeneic Stem Cell Transplantation

Allogeneic hematopoietic stem cell transplantation (alloHCT) is the only curative therapy of CLL. The CLL3X trial based on long-term observation of allografted patients found that reduced intensity conditioning (RIC) alloHCT can provide sustained disease control in patients with high-risk CLL, independent of TP53 status [69]. In this study, 33 of 44 patients (75%) with available long-term observation data were alive at the 6-year follow-up. Patients in CR with MRD-negativity 1 year after alloHCT have a 75% probability of remaining without relapse for at least 10 years. The development of RIC regimens has improved the tolerability of alloHCT in CLL with preserving graft versus leukemia effect.

Allogeneic stem cell transplantation should be considered in physically fit patients with refractory CLL or in those with a del17p/*TP53* mutation [70]. However, the decision to perform

alloHCT has recently become complicated by the appearance of effective targeted drugs. Newer therapies have disrupted prior paradigms, and alloHCT is now indicated to later stages of relapsed or refractory CLL. Recent guidelines developed by the Guidelines Committee of the American Society for Blood and Marrow Transplantation for standard-risk CLL patients recommend the use of allo-HCT only in the absence of a response, or in the case of any evidence of disease progression, following BCR inhibitor administration [71]. For patients with high-risk CLL, alloHCT is indicated after failing two previous lines of therapy and obtaining an objective response to BCR inhibitors or other new agents in clinical trials. In addition, patients with del17p or *TP53* mutation can be candidates for alloHCT after one previous line of therapy. Allogeneic hematopoietic stem cell transplantation is also suitable management for patients who did not achieve OR, or who progressed after BCR inhibitor administration but receive BCL-2 inhibitors, regardless of whether OR is achieved. A reduced-intensity conditioning regimen is indicated whenever possible. However, alloHCT is not feasible in many cases because of patient age or fitness level, the presence of comorbidities, or lack of a matching donor.

8.8 Conclusions

Despite recent progress in the treatment of CLL, almost all patients are destined to relapse. In patients with a disease-free interval longer than 24–36 months after milder chemoimmunotherapy, retreatment with the same first-line therapy is still a possible therapeutic option [72]. Patients resistant to the first-line therapy or with a short PFS, i.e., less than 24–36 months after immunochemotherapy, should be treated with BCR inhibitors (ibrutinib, idelalisib) and/or BCL-2 analogs (venetoclax). However, current guidelines now recommend kinase inhibitor therapy before repeat chemotherapy or chemoimmunotherapy in all patient subgroups.

Allogeneic stem cell transplantation should be offered to fit patients, especially those with a del(17p)/*TP53* mutation and who may be refractory to BCR inhibitors. Finally, despite the significant progress made in recent years, available therapies for refractory/relapsed CLL are only partially effective, and there is an obvious need to develop better strategies and new, more specific and active drugs. Patients with refractory disease should be treated within clinical trials whenever possible, with or without a transplantation option. Several ongoing clinical trials with novel therapies will further define the role of targeted agents in the treatment of patients with relapsed and refractory CLL.

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Part VI

Follow-up and Complications

9 Autoimmune Cytopenia in Chronic Lymphocytic Leukemia

Carol Moreno, Carolina Cuellar, and Eva Puy Vicente

9.1 Introduction

The presence of cytopenias of immune origin is not infrequent in chronic lymphocytic leukemia (CLL). These include autoimmune hemolytic anemia (AIHA), autoimmune immune thrombocytopenia (AITP), pure red cell aplasia (PRCA), and autoimmune granulocytopenia (AG). The most common is AIHA (about 7%) followed by AITP (<1 to 2%) either alone or in combination with AIHA (Evan's syndrome). AG is exceedingly rare. While the higher prevalence of autoimmune cytopenia in CLL patients as compared to the general population is well known, the pathogenesis underlying this phenomenon is unclear. Autoimmune cytopenias may occur either at diagnosis or over the course of the disease and can be triggered by treatment. In patients with CLL distinguishing immune cytopenia from cytopenia due to other causes (i.e., bone marrow infiltration, iron, B12 or folic acid deficiency, treatment-related bone marrow toxicity, hypersplenism) is important because of their different prognosis and management. In this chapter the pathophysiology and clinical aspects of autoimmune cytopenias in CLL are reviewed.

9.1.1 Pathophysiology of Autoimmune Cytopenias in CLL

The biological explanation for autoimmune cytopenia in CLL is complex and not completely understood, with non-neoplastic B cells, neoplastic B CLL cells, T cells, and microenvironment cells playing a role [1–3]. The B-cell response to antigens is mediated by the B-cell receptor (BCR). The analysis of the BCR in patients with CLL shows a stereotyped repertoire with an identical or almost identical sequence, suggesting selection of B cells with antigen binding sites of restricted structure [3, 4]. CLL cells, particularly those with unmutated IGHV genes, can present a highly polyreactive BCR which recognizes auto-antigens [5, 6]. Of note, the same antigens are recognized by "natural" antibodies known to be pathogenic in certain autoimmune diseases [7]. In line with this, a high prevalence of stereotyped B-cell receptor configuration (i.e., VH1-69) has been described in CLL patients with autoimmune cytopenia [8–10]. CLL cells can produce auto-reactive antibodies in vitro after stimulation [11, 12]. Although in

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rare instances CLL cells produce auto-reactive antibodies in vivo in sufficient quantity to cause clinical disease (e.g., cold agglutinin disease), in most cases autoimmune cytopenias associated with CLL are caused by polyclonal IgG antibodies produced by non-malignant B cells [13]. The capacity of CLL cells to function as antigen presenting cells is almost inexistent in vitro, the exception being red cell antigen Rh processing [14]. An alternative red cell antigen, B3, has been demonstrated to be processed by CLL cells, which are then able to provoke a T-cell response [15]. AIHA is more common in advanced CLL, where the spleen is heavily infiltrated by leukemic cells which brings CLL cells in close proximity to damaged red blood cells [16]. In this regard, the spleen also contains CD40 ligand-expressing T cells which in vitro are able to induce activation of CLL cells and improve antigen presentation [17]. It is also worth mentioning that CLL is associated with impairment of the innate immune system, with reduced activity of toll-like-receptors (TLR) (i.e., TLR4) having been associated with a high risk of autoimmune cytopenia in CLL [18–20].

On the other hand, CLL cells interact with T cells to modulate the immune environment, which may be important in permitting the development of autoimmunity. In addition, the imbalance of Tregs and Th17 has been associated with the development of autoimmune cytopenias in CLL [21]. These abnormalities are in keeping with T-cell numerical and functional defects that accompany CLL, including an increase in T cells, inversion of the CD4:CD8 ratio, production by CLL cells of the inhibitory cytokines IL-6, IL-10, TNF, and TGF-β, and alterations in T-cell cytoskeleton formation and vesicle transportation [22–25].

The role of MicroRNAs (miRNAs) in CLL pathogenesis is important (see Chap. 1). A number of miRNAs have been correlated with clinical characteristics. miRNAs also play a role in autoimmunity, including AIHA [26–28]. MiR-146b-5p targets CD80, a molecule associated with the B-T-cell synapse and in restoration of the antigen presenting cell capacity of CLL cells [29].

9.1.2 Correlation of CLL Clinical and Biological Characteristics with Autoimmune Cytopenias

Several reports have analyzed the association of autoimmune cytopenia and clinical and biological characteristics of CLL. Some of these data, however, come from retrospective studies and have limited clinical value. The association between advanced stage and autoimmunity has been reported in many studies, patients with active CLL showing a high prevalence of AIHA [16, 30, 31]. Other parameters associated with autoimmune cytopenia in CLL are older age, male gender, high white blood cell count, and duration of the disease [16, 31, 32]. The occurrence of autoimmune cytopenia has also been associated with unfavorable biomarkers such as unmutated IGHV genes, high ZAP70 and CD38 expression, increased serum beta-2 microglobulin levels, poor-risk cytogenetics [(del (17p), del (11q) and *TP53* gene mutations)] [32–37] (Table 9.1). Not surprisingly direct anti-globulin test (DAT) positivity may precede the development of a clinically apparent AIHA although altogether only a minority of patients with a positive develop AIHA [9, 32].

In most cases autoimmune cytopenia presents over the course of the disease and can be triggered by the treatment of the disease [38, 39] but it also can precede or be the feature leading to the diagnosis of monoclonal B-cell lymphocytosis or

Table 9.1 Prognostic factors correlated with the development of autoimmune cytopenia in CLL

Clinical prognostic factors
Advanced stage
Older age
Male
High white blood cell count
Short lymphocyte doubling time
Biological prognostic factors
Beta-2 microglobulin
High CD38 expression
High ZAP 70 expression
Unmutated IGHV genes
Poor-risk cytogenetics del (del 17p, del 11q)
BCR stereotyped (i.e., VH1-69)

Table 9.2 The incidence of autoimmune hemolytic anemia (AIHA) under fludarabine-based therapies

Population	Treatment regimen	AIHA incidence	Remarks
UK series, Myint et al. [46], $n = 52$	Fludarabine	11–23%	Unselected, advanced clinical stage, and heavily pretreated
UK CLL4 trial, Dearden et al. [32], $n = 777$	F vs. FC vs. Clb	12% chlorambucil	Selected, previously untreated
		11% fludarabine alone	
		5% FC	
MDA series, Borthakur et al. [47], $n = 300$	FCR	1–5% AIHA[a]	Selected, previously untreated
GCLLSG CLL 8 trial, Hallek et al. [48], $n = 409$ vs. 408	FC vs. FCR	<1% (FCR)—1% FC	Selected, previously untreated

F fludarabine, *C* cyclophosphamide, *R* rituximab, *Clb* chlorambucil, *AIHA* autoimmune hemolytic anemia
[a]1 patient developed immune thrombocytopenia (AITP) after completing FCR, and 1 pure red cell aplasia (PRCA)

CLL [33, 35, 40]. This indicates that immunophenotyping of mononuclear cells should be part of the diagnostic workup of patients with "idiopathic" autoimmune cytopenia [41].

9.1.3 Therapy-Associated Autoimmune Cytopenia

The relationship between treatment initiation and the development of AIHA was identified in seminal descriptions of CLL [42, 43]. In the 1990s, it was considered that treatment with purine analogs (particularly fludarabine) could be associated with a high frequency of autoimmune cytopenia as compared to that observed with alkylating agents [44–46]. This concept, however, was based on retrospective studies of highly selected and heavily pretreated patients. Currently, it is well established that purine analogs do not provoke a higher incidence of AIHA. Moreover, treatments combining purine analogs with other agents (e.g., cyclophosphamide [FC], FC + rituximab [FCR]) have in fact a "protective" effect over the development of AIHA, as further discussed below.

In the UK CLL4 trial the proportion of patients becoming DAT positive upon therapy was 14%, 13%, and 10% after chlorambucil, fludarabine, and fludarabine combined with cyclophosphamide, respectively. Likewise, AIHA was significantly lower in patients treated with fludarabine with cyclophosphamide (5%), compared to those receiving chlorambucil (12%) or fludarabine in monotherapy (11%). Of note patients with DAT positive but without active hemolysis were eligible for the study [32]. A retrospective study in a series of 961 patients also showed that the incidence of AIHA was slightly lower with fludarabine (4%) than chlorambucil (5%) [35]. Finally, in a phase II study, the incidence of AIHA reported with FCR was 5.8% [47]. Likewise, a low rate of AIHA (<2%) was observed in the German CLL 8 trial which investigated FC vs FCR in the treatment of CLL [48]. These results indicate that the risk of developing AIHA decreases with a more effective control of the leukemia, of which immune cytopenia is largely an epiphenomenon (Table 9.2).

9.2 Diagnosis of Autoimmune Cytopenia in Chronic Lymphocytic Leukemia

There are multiple, not mutually exclusive causes of cytopenia in CLL: bone marrow failure, autoimmunity, hypersplenism, chemotherapy, sepsis, iron, B12, or folic acid deficiency. The diagnostic workup should include the following laboratory tests: exam of a peripheral blood smear, hemoglobin and platelet counts, DAT, lactate dehydrogenase (LDH), bilirubin, haptoglobin, and reticulocyte count. Bone marrow aspirate/biopsy may provide important information about the origin of cytopenia [33, 35, 37, 49, 50].

Table 9.3 shows parameters that can be of help to distinguish cytopenias of immune origin vs. bone marrow infiltration. AIHA and/or ITP are occasionally preceded by a prior history of autoimmune cytopenia as compared to those in which cytopenia is due to bone marrow infiltration. In addition, its onset is usually abrupt, platelet or hemoglobin counts are very low, and there might be dissociation between hemoglobin level and platelet count which is infrequent in case of bone marrow infiltration. Moreover, indirect hemolytic signs (i.e., increase of bilirubin levels or LDH, low haptoglobin level) are usually present. In case of AIHA, a positive DAT is the most important diagnostic criterion. The DAT is usually positive for red cell-bound polyclonal IgG and/or C3. Cold agglutinin disease associated with IgM produced by the clonal CLL B cells has been reported but is extremely infrequent [51]. Serum LDH is less discriminating as it may be elevated due to active CLL. Moreover, DAT negative AIHA has been seen, particularly in association with therapy [47]. Reticulocytosis may not be observed in case of an overwhelming infiltration of bone marrow by leukemic cells or recent chemotherapy. Bone marrow examination is essential to distinguish between therapy related causes of cytopenia and immune cytopenias.

Thrombocytopenia in CLL is less common due to immune causes than to either splenomegaly and bone marrow failure or myelotoxicty related to therapy. In advanced disease, anemia usually occurs before thrombocytopenia [52], thus isolated thrombocytopenia is more likely to be immune in origin [53]. As per PRCA, its diagnosis should be considered in any patients with anemia and reticulocytopenia [54], and AG should be suspected in patients where there is isolated neutropenia without another cause being apparent [55].

Table 9.4 summarizes commonly used criteria to make the diagnosis of CLL-associated AIHA, AITP, PRCA, and AG.

Table 9.3 Some clues to make the differential diagnosis between autoimmune cytopenias and cytopenias due to bone marrow infiltration

	Immune	Bone marrow infiltration
Prior history of IC	Yes	No
Ongoing or recent therapy	No	Yes
Onset	Abrupt	Gradual
Plt count/Hb level	Very low	Moderately low
Bone marrow	Not massively infiltrated	Packed
	Glycophorin ++/ factor VIII	
Indirect signs hemolysis	Yes, *but not always*!	No
Spherocytes/ Large plts	Yes, not striking	No
Laboratory tests	AIHA: DAT(+)	DAT(−)
	ITP: No reliable tests	
Dissociated Hb/ plt count	Possible	No
Response to corticosteroids	Yes	

IC immune cytopenia, *AIHA* autoimmune hemolytic anemia, *AITP* immune thrombocytopenia, *DAT* direct antiglobulin test, *Plt* platelet, *Hb* hemoglobin

Table 9.4 Recommendations for the diagnosis of CLL-associated autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (AITP), pure red cell aplasia (PRCA), and autoimmune granulocytopenia (AG)

AIHA
–Positive DAT
–Reticulocytosis
–Elevated serum LDH
–Elevated serum indirect bilirubin
–Reduce serum haptoglobin
–Erythroid hyperplasia in bone marrow
DAT direct antiglobulin test, *LDH* lactate dehydrogenase
AITP
–Rapid and "unexplained" fall in the platelet count
–Augmented or normal number of megakaryocytes in the bone marrow
–No recent chemotherapy[a]
PRCA
–Severe normochromic-normocytic anemia
–Reticulocytopenia
–Erythroid precursors ≤1% of bone marrow cells
–No parvovirus B19 infection by polymerase chain reaction assay
–DAT negativity and no other indirect signs of hemolysis
–No recent chemotherapy[a]
AG
–Persistent and "unexplained" neutropenia
–Decreased or absent granulocyte precursors in bone marrow
–No recent chemotherapy[a]

[a]More than 4–8 weeks from the last chemotherapy infusion

9.3 Prognostic Significance of Immune Cytopenia in Chronic Lymphocytic Leukemia

The impact of autoimmune cytopenia on the prognosis of patients with CLL remains largely controversial probably because of the heterogeneity of the populations investigated, differences in study design, and data interpretation (Table 9.5). Ideally, prognostic significance of autoimmune cytopenia in CLL should be analyzed in each possible setting in which this complication can be observed: before diagnosis, at diagnosis, and over the course of the disease in either treated or untreated patients. As in most studies these different scenarios have not been separately analyzed, the impact of autoimmune cytopenia on the outcome of CLL patients will be here jointly discussed. In a retrospective analysis from a single institution that included 1203 patients with CLL, 52 of whom had AIHA, the occurrence of the autoimmune cytopenia was associated with active disease, but did not negatively influence survival [16], In contrast to a study from the Israeli CLL group including 213 patients with cytopenia, those patients who developed AIHA but no AITP had a worse survival compared to those without cytopenias [56]. As shown in Table 9.5 only a few studies have analyzed the outcome of patients with cytopenias according to their origin (immune vs infiltrative). In a cohort of 1750 patients, those with autoimmune cytopenia at the time of CLL diagnosis showed better outcome than those in whom cytopenia was due to bone marrow infiltration [33]. In addition, the development of autoimmune cytopenia at any time during the course of the disease did not result in a worse prognosis [50]. Similar results were observed in two more series [35, 37]. In the study from Barcelona Hospital Clínic based on 961 patients with CLL, those with autoimmune cytopenia had a better survival than those with cytopenia due to bone marrow infiltration. Likewise, the development of autoimmune cytopenia at any phase of the disease did not influence survival [35]. In contrast, in a population-based retrospective analysis of 754 patients with CLL

Table 9.5 Prognostic significance of autoimmune cytopenias in CLL

References	Population	AIC—Impact on survival	Binet stage C "immune" vs "non-immune"
Mauro et al. [16]	Single institution, *n* = 1203 (52 AIHA)	No impact	NR
Kyasa et al. [40]	Single institution, *n* = 132 (6 AIHA, 5 ITP, 1 PRCA)	No impact	NR
Zent et al. [33]	Single institution, *n* = 1750 (41 AIHA, 35 ITP, 9 PRCA, 3 AIG)	No impact	Yes (better outcome)
Dearden et al. [32]	UK LRF CLL4 trial, *n* = 777 (77 AIHA)	Negative impact	NR
Moreno et al. [35]	Single institution, *n* = 961 (49 AIHA, 20 ITP, 1 Evan's syndrome)	No impact	Yes (better outcome)
Zanotti et al. [36]	Single institution, *n* = 290 (31 AIHA, 10 ITP, 4 Evan's syndrome, 1 PRCA)	Negative impact	NR
Shvidel et al. [56]	Israeli CLL group, *n* = 1518 (80 AIHA, 31 ITP, 11 Evan's syndrome)	Negative impact (patients with AIHA but not patients with ITP)	NR
Ricci et al. [58]	Single institution, *n* = 146 (9 AIHA)	No impact	NR
Alzaki et al. [57]	Providence Health Care, Canada, *n* = 754 (16 AIHA, 8 ITP, 5 Evan's syndrome, 1 PRCA)	Negative impact	No impact
Visco et al. [37]	Multicenter series, *n* = 86 (11 AIHA, 12 ITP, 4 Evan's syndrome)	Negative impact	Yes (better outcome)
Quinquenel et al. [9, 68]	Single institution, *n* = 378 (20 AIHA)	Negative impact	NR

AIC autoimmune cytopenia, *AIHA* autoimmune hemolytic anemia, *AITP* immune thrombocytopenia, *PRCA* pure red cell aplasia, *NR* not reported

from Canada, AIHA had a negative impact on survival, the prognosis of patients with anemia being independent of the origin of cytopenia, autoimmune, or infiltrative [57].

The prognostic significance of DAT has been analyzed in several studies. In the UK CLL4 trial, DAT positivity at the time of treatment correlated with poor outcome, although only few patients developed overt AIHA. In addition, the occurrence of a positive DAT and/or AIHA was associated with a worse overall survival [32]. Similar results were found in two single institution cohorts, in which DAT positivity at any time during the course of the disease was associated with poor outcome [9, 58]. Interestingly, the adverse prognostic of DAT positivity was maintained in patients with poor prognosis as defined by unmutated IGHV [58].

9.4 Management of Autoimmune Cytopenias in CLL

Treatment of autoimmune cytopenia in patients with CLL is largely based on retrospective studies, experts' opinion, and consensus guidelines. At first, important consideration is that treatment modality will depend on whether the disease is active and requires therapy at the time the diagnosis of autoimmune cytopenia is made. If autoimmune cytopenia is observed in the context of quiescent CLL, the treatment should be the same as in idiopathic immune cytopenia. Although rare, AIHA may be due to cold antibodies; these patients usually respond poorly to corticosteroids and the preferred therapeutic option is an anti-CD20 monoclonal antibody alone or in combination with steroids [59, 60]. For AIHA due to warm antibodies, initial therapy is high-dose corticosteroids followed by anti-CD20 monoclonal antibody in case of treatment failure [61–63].

In a retrospective analysis including 20 patients with autoimmune cytopenia including AIHA, AITP, and PRCA with progressive CLL, therapy with rituximab in combination with cyclophosphamide, vincristine, and prednisone (R-CVP) proved to be an effective treatment and in 19 out of the 20 patients the autoimmune cytopenia responded. Nevertheless, the duration of response was short and recurrence of autoimmune cytopenia was observed in 6 patients who required maintenance therapy. Moreover, CLL responses were seen in 17 patients (9 CR, 8 PR) [64]. A regimen consisting of rituximab combined with cyclophosphamide and dexamethasone (RCD) has shown good results, the overall response rate being of 83–100%, and the median duration of response 24 months. In addition, although relapses are frequent retreatment with the same regimen is effective [65–67]. Interestingly, in one of these studies the duration of response was longer when autoimmune cytopenia occurred early during the CLL course (<3 years) [67]. In a retrospective study, the French CLL Study group reported its experience with the BR regimen (bendamustine and rituximab) in a series of 26 patients with active CLL and AIHA, 88% of them with a prior history of AIHA and who had been previously treated with RCD and corticosteroids. In this poor-risk population BR resulted in an overall response rate of 81% for AIHA and 77% for CLL [68]. The effectiveness of anti-CD20 monoclonal antibodies is most likely due to their antileukemic rather than immunosuppressive effect. If there is no response to these approaches, alternative immunosuppression (e.g., cyclosporine, cyclophosphamide, or azathioprine) may be considered [69, 70]. Finally, splenectomy can be useful in individual cases but carries morbidity and mortality, and has almost been abandoned as treatment of refractory AIHA in patients with CLL. Supportive care should include red blood cells transfusion as clinically indicated and folic acid. Of interest, cases of parvovirus B19 infection causing pure-red blood cells aplasia in the context of AIHA have been reported [71–73], a possibility to be kept in mind. Failure of autoimmune cytopenia to respond to conventional treatment is an indication for anti-CLL therapy [74]. Table 9.6 summarizes the most employed therapeutic regimens to treat autoimmune cytopenia in CLL and a treatment algorithm is shown in Fig. 9.1.

Table 9.6 Therapeutic regimens used to treat CLL-associated autoimmune cytopenia

N° AIHA cases, reference	Therapy	Outcome	Relapse	Survival
8 AIHA, Gupta et al. [65]	RCD	8 CR (5 DAT negative)	5 relapses after a median of 13 months, second remission following RCD	6 alive. 2 died of progressive CLL Median follow-up 21 months
14 AIHA, D'Arena et al. [61]	R monotherapy	22% CR, 50% PR	NR	8 alive (6 transfusion-free) Mean follow-up of 17 months
18 AIHA, 1 AITP, 2 Evan's syndrome, Kaufman et al. [66]	RCD	100% CR of AIHA (10 DAT negative)	12 AIHA, 1 ITP	OS 70 months
26 AIHA, 9 AITP, 8 Evan's syndrome, 5 PRCA, Michallet et al. [67]	RCD	AIHA: 89.5% ORR 83% CR CLL: 95% ORR 35% CR	19 (39.6%) (CLL progression) 8 second line RCD → 7 CR (87.5%)	OS 52 months
20 AIHA, PRCA, and/or AITP, Bowen et al. [64]	R-CVP	IC: 14 CR, 5 PR CLL: 9 CR, 8 PR	9 (3 PR, 6 CR)	Median OS 61.2 months (follow-up 15–30 months)
33 R/R CLL, Robak et al. [62]	Ofatumumab	ORR 50%		Not specified follow-up
1 AIHA, Nader et al. [63]	Ofatumumab	CR	CLL progressed during treatment	Follow-up 3 months
26 AIHA, Quinquenel et al. [9, 68]	BR	ORR: 81% AIHA, 77% CLL CR: 31% AIHA, 58% CLL	10 relapses	OS 80% at 12 months. 9 died Median follow-up 30.7 months
1 AIHA, Manda et al. [81]	Ibrutinib	CR		No follow-up
3 AIHA, 4 AITP, 4 Evan's syndrome, 1 PRCA, 1 AIHA/AITP and PRCA, Vitale et al. [76]	Ibrutinib	8 CR and 5 PR	No relapses	Follow-up 4–33 months
1 AIHA, Cavazzini et al. [92]	Ibrutinib	CR		No follow-up

RCD rituximab, cyclophosphamide, and dexamethasone, *CVP* cyclophosphamide, vincristine, and prednisone, *BR* bendamustine plus rituximab, *AIHA* autoimmune hemolytic anemia, *PRCA* pure red cell aplasia, *AITP* immune thrombocytopenia, *IC* immune cytopenia, *R/R CLL* relapsed/refractory chronic lymphocytic leukemia, *ORR* overall response rate, *CR* complete remission, *PR* partial remission, *OS* overall survival

9.4.1 Autoimmune Cytopenias and Targeted Therapies

A major innovation in the treatment of CLL has been the introduction of agents specifically targeting the B-cell antigen receptor (BCR inhibitors—BCRi) or the antiapoptotic protein BCl-2 (BCL-2 inhibitors BCL2i). A highlighting retrospective analysis from Ohio State University included 301 patients with relapsed/refractory CLL treated with ibrutinib within four clinical trials between 2010 and 2014 [75]. In three of these trials patients received ibrutinib as monotherapy while in the remaining one ibrutinib was given in combination with ofatumumab. Twenty-six percent (78/301) of the patients had experienced an episode of autoimmune cytopenia prior starting ibrutinib (44 (56%) had AIHA, 25 (32%) had AITP, 8 (10%) had both AIHA and AITP either concomitantly or sequentially, and 1 (1%) had PRCA) and 22 patients were given immunosuppressive agents (e.g., prednisone), in all patients

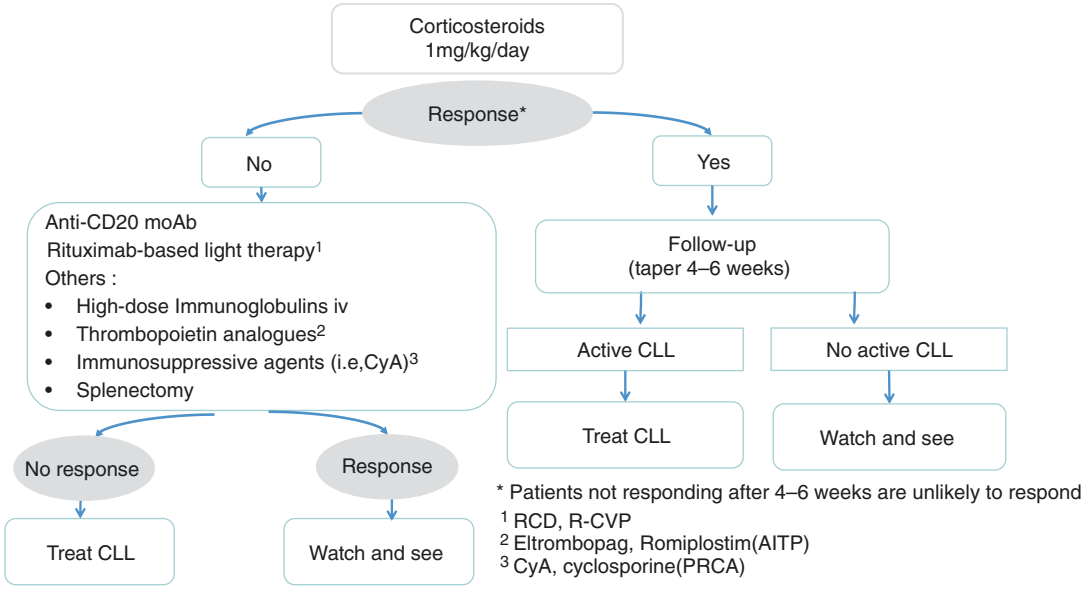

Fig. 9.1 Treatment algorithm for autoimmune cytopenia in CLL

autoimmune cytopenia being under control when ibrutinib was started. Interestingly, 86% (19/22) discontinued immunosuppression at a median time of 4.7 months after starting ibrutinib, and only one patient relapsed requiring high doses of corticosteroids to treat the hemolysis while ibrutinib was withdrawn. On the other hand, in this poor-risk CLL population, only six patients developed emergent autoimmune cytopenia which corresponded to an estimated incidence rate of 13 episodes for every 1000 patient-years of ibrutinib treatment. Furthermore, the six cases who presented autoimmune cytopenia (4 AIHA and 2 AITP) were treated with corticosteroids, intravenous immunoglobulins or both; three of them were able to continue ibrutinib without an exacerbation of the autoimmune process [75]. Likewise, the MD Anderson Cancer Center group reported its experience in 13 patients with signs of autoimmune cytopenia (3 AIHA, 3 AITP, 4 Evans' syndrome, and 2 had prior history of PRCA), 7 had autoimmune cytopenia controlled with no treatment, 6 were receiving autoimmune therapy but 3 had active not controlled autoimmune cytopenia at the time of ibrutinib initiation. Nine out of the 13 patients were treated within clinical trials. During the first weeks, a flare of the autoimmune process was observed in 9 of them, but all patients were successfully managed by continuation of ibrutinib and the addition of therapy (i.e., corticosteroids, iv immunoglobulins, eltrombopag) to control the immune process [76]. Interestingly, the autoimmune cytopenia was also controlled in the three patients who showed active autoimmune cytopenia when ibrutinib was started.

In the RESONATE trial, including 386 relapsed/refractory and heavily pretreated patients, no patient on the ibrutinib arm ($n = 195$) developed autoimmune cytopenia as compared to 4 of 191 patients allocated to the ofatumumab control arm; past history of AIHA and/or AITP was recorded in 38 and 42 patients in ibrutinib and ofatumumab arms, respectively. Of note, ongoing AIHA and/or AITP receiving autoimmune therapy at the time of initiating antileukemic treatment was present in 29 patients in the ibrutinib arm and in 16 patients allocated to ofatumumab [77]. Finally, in 269 elderly patients with CLL comparing treatment with ibrutinib vs chlorambucil as front-line therapy, no cases of autoimmune cytopenia were observed in 136 subjects receiving ibrutinib compared to 2% in 133 patients who received chlorambucil [78]. Taken together these results indicate that the prevalence of autoimmune cytopenia under ibrutinib is low and that a past history of autoimmune cytopenia or DAT

positivity does not predict autoimmune cytopenia upon ibrutinib therapy. The prevalence of autoimmune cytopenia under treatment with second generation of BTK inhibitors (i.e., acalabrutinib) has been also low. In a phase I–II trial, among 61 patients with relapsed CLL treated with acalabrutinib, only 2 cases developed AIHA [79]. As per BCL2i, venetoclax, 13 cases with emergent autoimmune cytopenia (8 AIHA and 5 AITP) have been reported in a phase II multicenter study including 101 relapsed/refractory CLL patients [80]. There is no data on the incidence of autoimmune cytopenia in patients treated with PI3K inhibitors (i.e., idelalisib).

As the prevalence of adverse events in routine clinical practice may be different to that observed in clinical trials, "real-life" studies are of interest. A few reports based on small number of patients and likely suffering from selection bias show contradictory results. While some reports suggest that ibrutinib can be safely given in patients with prior history of AIHA [81, 82], others communicate emergent AIHA under ibrutinib therapy [83]. Unfortunately, in several reports on ibrutinib efficacy and toxicity in a "real-life" scenario, the prevalence of autoimmune cytopenia has not been reported [84–87], except in one study in which among 286 patients with CLL treated with ibrutinib, 4% of patients (11/286) developed autoimmune cytopenia including AIHA (n = 4), AITP (n = 4), PRCA (n = 2), and AG (n = 1). Of note 75% (8/11) of patients were able to continue ibrutinib therapy while only 3 discontinued treatment because of the autoimmune cytopenia [88]. In summary, current evidence indicates that the prevalence of AIHA and other immune cytopenias in patients with CLL treated with novel agents is comparable to that observed in the general population of persons with CLL.

Conversely, small molecules could be effective to treat autoimmune disorders. Ibrutinib inhibits the production of autoantibodies in murine models of autoimmunity [89]. In addition, through the inhibition of BTK, ibrutinib targets not only B cells but also other effector cells (i.e., macrophages, monocytes, and mast cells) involved in several autoimmune disorders [90]. Furthermore, ibrutinib inhibits the interleukin-2-inducible kinase and can promote a shift from Th2-towards Th1-polarized immunity that is less favorable for the development of AIHA [91]. Table 9.7 summarizes main studies which have

Table 9.7 The incidence of autoimmune cytopenia under ibrutinib

Population	History of IC, n	Ongoing IC, n[a]	IC emergent, n	IC resolved, n	IC relapsed, n	Remarks
Ohio State, n = 301 pts	78	22[b]	6	19[b]	1	Selected population, relapsed/refractory patients
RESONATE trial (PCYC 1112), n = 386 pts	38[c]	41	0	1	0	Selected population, relapsed/refractory patients
MD Anderson Cancer Center, n = 13[d]	NA	13[e]	NA	9	0	Selected population, relapsed/refractory patients
Manda et al. [81], n = 1 AIHA	1	1	NA	1	0	Case report, AIHA refractory
Molica et al. [82], n = 1 AIHA	1	0	NA	1	1^	Case report, AIHA refractory
Rider et al. [83], n = 1 AIHA	0	0	1	0	0	Case report

IC immune cytopenia
[a]Ongoing IC at the time of starting ibrutinib
[b]Included patients with AIHA, AITP or both, and PRCA
[c]Included patients with AIHA and AITP
[d]Included patients with AIHA, AITP or both, and PRCA
[e]A flare of autoimmune cytopenia was detected in 9 patients but resolved or controlled in all; ^AIHA relapsed when the ibrutinib was withdrawn due to an infection episode

analyzed the incidence of autoimmune cytopenia under ibrutinib therapy.

Outside clinical trials, few case reports of autoimmune cytopenia associated with CLL successfully treated with ibrutinib have been reported. In one of those, an impressive activity of ibrutinib was observed in a patient with poor prognosis (i.e., del 17(p)) who presented with AIHA refractory to four prior lines of therapy (corticosteroids, cyclophosphamide, vincristine, and rituximab). Interestingly, the hemolytic process was controlled after 3 weeks of starting ibrutinib with complete resolution afterwards [81]. Similarly, another case with del (17p) and uncontrolled and refractory AIHA to multiple lines of therapy has been reported. The initiation of ibrutinib was followed by a quick stabilization of the autoimmune cytopenia and complete resolution after 6 months of ibrutinib treatment [92]. Patients with CLL treated with BCRi, particularly ibrutinib, and BCL2i do not present a higher risk of presenting autoimmune cytopenia and that these agents can be safely administered to patients with prior history of autoimmune cytopenia. Moreover, there is proof that ibrutinib can be effective in the treatment of autoimmune cytopenia.

9.5 Conclusions

The link between CLL and autoimmune cytopenia is clearly established by clinical experience and epidemiological studies. In vivo and in vitro research continues to increase our understanding of the complex interactions between the malignant CLL cells and the normal cellular and humoral immune systems that lead to this complication. The clinical impact of autoimmune cytopenias on patient's outcome is still controversial. Regarding therapy, it is recommended to treat first the autoimmune process with immunosuppression, basically corticosteroids followed by anti-CD20 monoclonal therapy and other alternative immunosuppressive agents if needed. The lack of response to these agents is an indication for CLL therapy. Ibrutinib is not associated with a higher prevalence of autoimmune cytopenias and in fact is a potentially treatment alternative approach for them. Large and well-conducted studies and meta-analysis are needed to better ascertain the prognostic impact of autoimmune cytopenias in CLL and to establish evidence-based treatment algorithms.

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Richter Syndrome

10

Adalgisa Condoluci and Davide Rossi

10.1 Definition and Morphology

Richter syndrome (RS) is defined as the development of an aggressive lymphoma in patients with a previous or concomitant diagnosis of chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL). Two pathologic variants of RS are currently recognized, namely the diffuse large B-cell lymphoma (DLBCL) variant and the Hodgkin lymphoma (HL) variant [1].

Morphologically, the DLBCL-type RS consists in confluent sheets of large neoplastic B lymphocytes resembling either centroblasts (60–80% of cases) or immunoblasts (20–40% of cases) [2–4]. CLL transformation should be differentiated from CLL progression. From a pathological standpoint, CLL progression is sometimes associated with an increase in size and proliferative activity of the CLL cells, as well as an expansion of the proliferation centers in the lymph nodes which may become confluent and enriched of proliferating cells [1]. Such, progressive CLL cases have been heterogeneously defined as "aggressive" CLL or "accelerated" CLL and should be distinguished from RS as they have an outcome intermediate between typical CLL and classic RS [5]. Since the current WHO Classification of Haematopoietic and Lymphoid Tissues does not provide criteria supporting the differentiation between "accelerated" CLL and RS, such distinction is based on the individual interpretation and expertise of the pathologist. Criteria for differentiating RS from "accelerated" CLL have been proposed [6], and include the occurrence of: (1) tumor of large B-cells with nuclear size equal or larger than macrophage nuclei or more than twice as normal lymphocyte; and (2) diffuse growth pattern of such large cells (not just presence of small foci) (Fig. 10.1). By applying these criteria, up to 20% of cases diagnosed as RS are actually better classified as "accelerated" CLL [6]. Phenotypically, tumor cells of the DLBCL-type RS are CD20 positive, while CD5 is expressed in only a fraction (~30%) of cases, and CD23 expression is even more rare (~15% of cases) [2]. Based on immunophenotypic markers, most cases of the DLBCL-type RS (90–95%) have a post-germinal center phenotype (IRF4-positivity) whereas only 5–10% display a germinal center phenotype (CD10 expression) [2]. Based on the analysis of immunoglobulin genes, most of the DLBCL-type RS (~80%) are clonally related to the preceding CLL phase, thus representing true transformations [2, 4].

Diagnosis of the HL variant of RS requires classical Reed–Sternberg cells showing a CD30 positive/CD15 positive/CD20 negative phenotype in an appropriate polymorphous background

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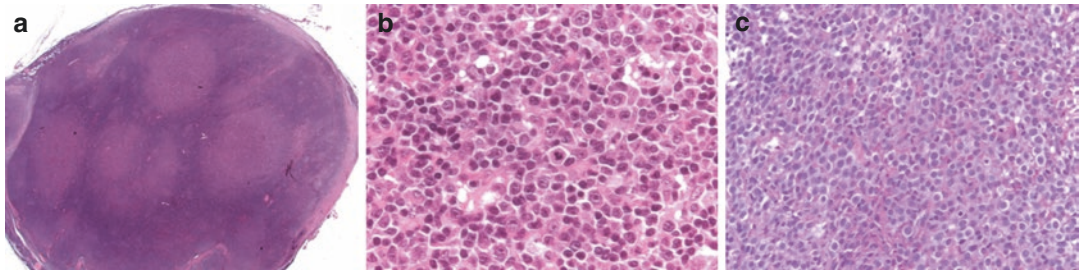

Fig. 10.1 Representative case of lymph node involvement by prolymphocytic progression (**a**, **b**). At low (**a**) and high (**b**) magnification the tumor shows the typical pattern with expanded proliferation centers wider than a 20× field (clear areas) surrounded by the small lymphocytic component (dark areas) (hematoxylin-eosin stain). (**c**) Representative case of DLBCL-type Richter transformation, with involvement of the lymph node by large immunoblastic cells

of small T-cells, epithelioid histiocytes, eosinophils, and plasma cells [7]. The presence of Reed–Sternberg-like cells atypically expressing both CD30 and CD20 but lacking CD15 in the background of CLL does not qualify for the diagnosis of HL [7]. The vast majority of cases of the HL-type RS are EBV positive and harbor distinct immunoglobulin rearrangements compared to the paired CLL, thus representing *de novo*, EBV-driven lymphomas arising in a CLL patient [7].

10.2 Pathogenesis of DLBCL-Type Richter Syndrome

The molecular profile of the DLBCL-type RS is heterogeneous, lacks a unifying genetic lesion, and does not overlap with the genetics of *de novo* DLBCL. Indeed, transformed DLBCL lacks molecular lesions in signaling pathways and B-cell differentiation programs that are otherwise commonly targeted in *de novo* DLBCL. The DLBCL-type RS shares with other transformed lymphomas (i.e., transformed follicular lymphoma) a common molecular signature characterized by lesions affecting general regulators of tumor suppression, cell cycle, and proliferation [4, 8, 9]. Deregulation of these programs conceivably accounts for the aggressive clinical phenotype of DLBCL-type RS that combines chemoresistance and rapid disease kinetics. Genetic lesions of DLBCL-type RS recurrently target the *TP53*, *NOTCH1*, *MYC*, and *CDKN2A* genes (Fig. 10.2) [4, 8, 9].

The most frequently affected gene in the DLBCL-type RS is *TP53* that harbors either mutation or deletion in ~60% of cases [4]. *TP53* abnormalities are generally acquired at the time of transformation, suggesting that they have been selected at the histologic shift (Fig. 10.3) [4]. *TP53* is a master regulator of the DNA-damage-response pathway which leads to cell apoptosis if activated. Consistently, it has a central role in mediating the antiproliferative effect of chemotherapies, and its loss may explain the chemorefractory phenotype generally shown by DLBCL-type RS.

CDKN2A deletions are found in ~30% of cases (Fig. 10.3). *CDKN2A,* also known as p16, is a negative regulator of cell cycle transition from G1 phase to S phase [8, 9]. Cell cycle deregulation by *CDKN2A* may explain the rapidly progressive behavior of DLBCL-type RS.

MYC genetic alterations sustain ~40% of DLBCL-type RS (Fig. 10.3) [4, 10]. *MYC* is involved in a transcription regulating network which is balanced by its antagonists *MAX* and *MGA*, with *MYC-MAX* heterodimers sustaining gene transcription and *MAX-MGA* heterodimers suppressing *MYC*-dependent gene expression. The *MYC* network is generally deregulated by somatic structural alterations of *MYC* (~30% of cases), including translocations juxtaposing *MYC* to immunoglobulin *loci*, gain/amplification at 8q24, and point mutations [4, 8, 9, 11]. *MYC* activation is also sustained by truncating mutations and deletions of *MGA* in ~10% of DLBCL-type RS [10], and by mutations affecting *MYC*

Fig. 10.2 Genes mutated in DLBCL-type RS. The word cloud shows the molecularly deregulated genes in DLBCL-type RS according to Fabbri et al. [9]. The size of the font is proportional to the mutation frequency

trans-regulatory factors as *NOTCH1* (~30% of DLBCL-type RS) [12, 13].

The DLBCL-type RS shows biased usage of the subset 8 configuration in the B-cell receptor (BCR), supporting a role of BCR signaling in transformation (Fig. 10.3) [3]. The strong and unlimited capacity of CLL harboring this BCR configuration to respond to multiple auto-antigens and immune *stimuli* from the microenvironment may explain the particular aggressiveness of CLL harboring subset 8 BCR and their increased propensity to transform into RS [14].

EBV infection has been suggested as a pathogenetic trigger of DLBCL-type RS. The observation that the overwhelming majority (85-100%) of DLBCL transformed from CLL does not carry EBV infection in the malignant cells, however, does not favor this hypothesis [4].

10.3 Prevalence and Risk Factors of DLBCL-Type Richter Syndrome

Prevalence of DLBCL-type RS is highly variable (1–23%) and depends on whether the analysis is restricted to biopsy-proven cases or also includes patients with clinically suspected transformation (Table 10.1) [15–38]. In addition, the diagnostic aggressiveness in case of rapidly progressive lymphadenopathy can influence DLBCL-type RS prevalence. Among CLL patients included in clinical trials, the prevalence of DLBCL-type RS ranges from 2 to 7%. However, also these rates must be interpreted with caution because they are derived from selected patients who require treatment, fit the eligibility criteria for trial participation, and in which the therapy used may have influenced the risk of transformation (Table 10.1) [15–38]. By taking into account such limitations, the more reliable estimate of the incidence rate of the DLBCL-type RS is ~0.5% per year of observation [3, 26].

Early recognition of RS transformation may be clinically useful in order to avoid the exposure of patients to multiple lines of therapy that, being targeted to CLL progression, are of little efficacy for the transformed clone. This concept prompts the need for a close monitoring of CLL patients harboring risk factors of RS development. The sole risk factor for the DLBCL-type RS that has been validated in independent CLL cohorts is the mutational status of *NOTCH1*. CLL patients presenting with *NOTCH1* mutations have a

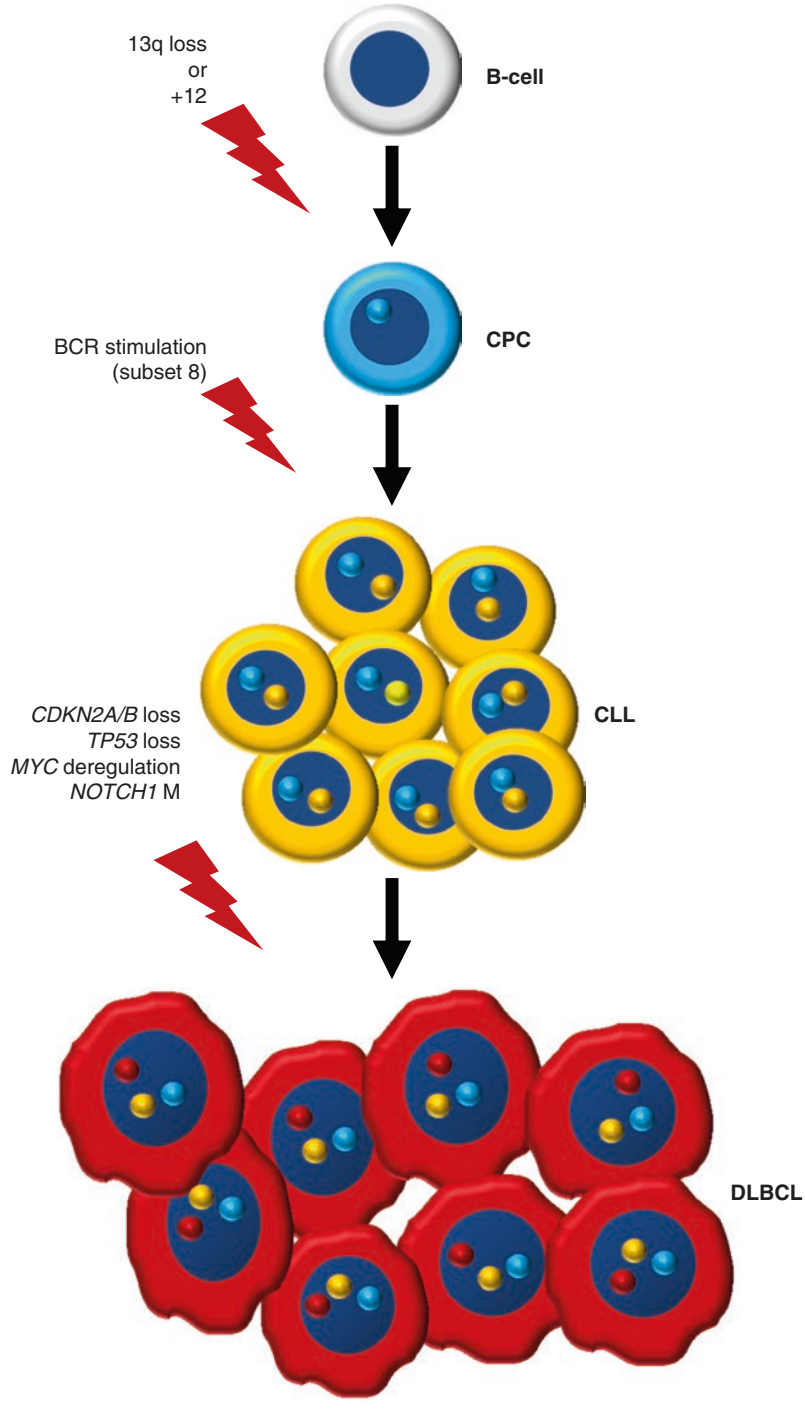

Fig. 10.3 Molecular lesions contributing to CLL transformation into DLBCL. Evolution steps from normal B-cells to common progenitor cell (CPC), CLL, and transformed DLBCL are shown. Molecular lesions associated with each step of the clonal evolution are highlighted

Table 10.1 Summary of the published reports assessing the frequency of Richter syndrome transformation[a]

Reference	Study design	CLL patients included in the study	Study population	Treatment	Patients who developed RS	RS prevalence (%)	RS type
Maddocks-Christianson [20]	Retrospective	962	Unselected	na	14	1	Any
Robak [18]	Retrospective	1487	Unselected	na	15	1	Any
Byrd [29]	Clinical trial	391	Relapsed	Ibrutinib, ofatumumab	4	1	Any
Catovsky [22]	Clinical trial	777	Progressive untreated	Clb, F, FC	13	2	Any
Mauro [17]	Retrospective	1011	Unselected	na	22	2	Any
Parikh [26]	Observational	1641	Unselected	na	37	2	DLBCL
Eichhorst [34]	Clinical trial	561	Untreated, 17p non-deleted	FCR, BR	13	2	Any
O'Brien [30]	Clinical trial	29	Progressive untreated	Ibrutinib	1	3	Any
Tsimberidou [21, 66]	Retrospective	3986	Unselected	na	148	4	Any
Fischer [35]	Clinical trial	817	Progressive untreated	FC, FCR	33	4	Any
Jain [33]	Clinical trial	127	Relapsed/refractory	Ibrutinib	7	5	Any
Alipour [23]	Retrospective	465	Unselected	na	24	5	Any
Tabuteau [16]	Retrospective	620	Unselected	na	37	6	Any
Farooqui [32]	Clinical trial	51	17p deleted	Ibrutinib	3	6	Any
Keating [15]	Clinical trial	174	Progressive untreated	F	13	7	Any
Solh [27]	Clinical trial	521	Progressive untreated	Clb, F, Clb + F	34	7	Any
Mato [36]	Retrospective	178	BCRi treated	Ibrutinib, idelalisib	13	7	Any
Byrd [25]	Clinical trial	85	Relapsed/refractory	Ibrutinib	7	8	Any
Benjamini [28]	Clinical trial	234	Progressive untreated	FCR	21	9	Any
Rossi [3]	Retrospective	783	Unselected	na	69	9	DLBCL
Rossi [24]	Retrospective	185	Unselected	na	17	9	DLBCL
Thornton [19]	Retrospective	101	Unselected	na	12	12	Any
Roberts [37, 43]	Clinical trial	116	Relapsed/refractory	Venetoclax	18	16	Any
Strati [31]	Clinical trial	63	17p deleted	Heterogeneous	15	23	Any
Parikh [65]	Observational	3887	Unselected	na	26	0.7	HL
Anderson [42]	Clinical trial	67	Relapsed/refractory, 17p del	Venetoclax	17	25	Any

[a]*CLL* chronic lymphocytic leukemia, *RS* Richter syndrome, *DLBCL* diffuse large B-cell lymphoma, *HL* Hodgkin lymphoma, *na* not available, *Clb* chlorambucil, *F* fludarabine, *C* cyclophosphamide, *R* rituximab, *BCRi* B-cell receptor pathway inhibitors

significantly higher cumulative probability of developing DLBCL-type RS (45%) compared to CLL without *NOTCH1* mutations (4%) [38–40].

10.4 Role of CLL Treatment in the Development of DLBCL-Type Richter Syndrome

The exposure to a prior CLL treatment has been claimed as a risk factor for the development of DLBCL-type RS, though evidence is still conflicting. The incidence rate of DLBCL-type RS does not significantly differ on whether the patient has been treated with chlorambucil, fludarabine, or fludarabine plus cyclophosphamide [22, 27]. The incidence of DLBCL-type RS seems to be lower in patients treated with rituximab, fludarabine, and cyclophosphamide when compared to patients treated with fludarabine and cyclophosphamide alone, suggesting a protective role of rituximab against RS [35].

Changes in the treatment scenario of CLL might change the epidemiology, biology, and genetics of RS. Though the limited follow-up prevents definitive conclusions, the rate of transformation among relapsed/refractory CLL treated with ibrutinib, idelalisib, or venetoclax seems to be comparable to that of historical controls treated with chemo/chemoimmunotherapy [25, 29–33, 36, 37]. Consistent with the lack of a specific contribution of novel agents to RS development, RS occurred at similar rates among relapsed CLL randomized to receive ibrutinib vs ofatumumab and idelalisib vs rituximab [29, 41]. Given the lack of data comparing treatment with venetoclax vs non-novel agent treatment, it is unknown whether venetoclax might modify the epidemiology of RS. In a small cohort of heavily pre-treated CLL patients harboring 17p abnormalities and who received venetoclax, ~25% progressed with histologically confirmed RS [42]. In a broader population of less heavily pre-treated patients, the development of RS during venetoclax was far less common (12%) [43]. These data indirectly suggest that the prevalence of RS in venetoclax treated patients is proportional to that expected in the same population when chemoimmunotherapy is used. In venetoclax trials, RS occurred after a short time frame (generally within 1 year from treatment start), suggesting that some patients entered the trial with pre-existing and undiagnosed RS [42]. The biology of RS after ibrutinib or other novel agents is largely unknown, though morphological and immunophenotypic features of DLBCL, highly aggressive outcome, and disease unresponsiveness to chemoimmunotherapy have been reported for this condition [36, 44]. At variance with CLL progression, genetic findings indicate that RS developed under ibrutinib lacks resistance mutations of the *BTK* and *PLCG2* genes [45].

10.5 Approach to Richter Syndrome Diagnosis

The clinical suspicion of RS transformation should arise in CLL patients developing physical deterioration, fever in the absence of infection, rapid and discordant growth of localized lymph nodes, and/or sudden and excessive rise in lactate dehydrogenase (LDH) levels. The specificity of these clinical signs for RS transformation is only 50–60%. In the remaining cases, the histopathologic assessment can either show progressive CLL, "accelerated" CLL, or even solid cancer [46]. In some cases, RS may arise in extra nodal sites, and it should be included in the differential diagnosis if an extra nodal mass develops in a CLL patient.

In case of clinical suspicions of transformation, the ^{18}FDG PET/CT characteristics of the lesion, in particular the standardized uptake value (SUVmax), may guide the choice of whether to perform a biopsy, since sites affected by RS are expected to have SUVs overlapping with those of *de novo* DLBCL [46–48]. A SUV greater than 5 has a high sensitivity (91%) for detecting RS transformation, but it has low specificity (80%), since it may also highlight lymph nodes with expanded proliferation centers, infections, or metastases of solid tumors. The main contribution of ^{18}FDG PET/CT in RS diagnosis relies on its high (97%) negative predictive value, meaning

that in the presence of a negative ^{18}FDG PET/CT, the final probability of RS transformation is only 3% [46]. Consistently, if the ^{18}FDG PET/CT is negative, biopsy can be avoided.

Histologic documentation is mandatory to diagnose RS. An open biopsy is considered the gold standard for RS diagnosis, since samples obtained with fine needle biopsy or aspiration may not be representative of the pathologic architecture of the tumor, especially in cases where the sheets of transformation are admixed to small cells. Furthermore, fine needle biopsy of an enlarged proliferation center that may be occasionally observed in lymph nodes of progressive or "accelerated" CLL may give rise to false positive misdiagnosis of RS transformation [49]. Since RS is often restricted to one single lesion at transformation, any biopsy aimed at exploring whether RS has occurred should be directed at the index lesion (i.e., the lesion with the largest diameter by imaging, the lesion showing the most rapid kinetics of progression, and/or the lesion displaying the most avid ^{18}FDG uptake at PET/CT).

10.6 Treatment Options for DLBCL-Type Richter Syndrome

RS is always an indication for treatment, and watch and wait is not an option. Patients who are unfit for an active treatment should be considered for palliation.

Chemotherapy Approaches Chemotherapy regimens commonly used to treat aggressive and high grade B-cell non-Hodgkin lymphomas have been investigated in DLBCL-type RS. R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) has shown a response rate of 67% (CR 7%), with a median progression-free survival (PFS) of 10 months and a median overall survival (OS) of 21 months [50]. The treatment-related mortality of R-CHOP is low (3%), and hematotoxicity (65% of patients) and infections (28% of patients) are the most common adverse events of this regimen (Table 10.2) [50]. Ofatumumab, an anti-CD20 monoclonal antibody with greater complement-mediated cytotoxicity than rituximab, has the potential of inducing apoptosis in tumor cells harboring *TP53* abnormalities, which is a common genetic event in DLBCL-type RS. However, the substitution of rituximab with ofatumumab as anti-CD20 monoclonal antibody within the CHOP schema does not improve response rate and survival when compared to historical cohorts treated with R-CHOP [51]. R-EPOCH (rituximab, etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin) is used in high grade B-cell lymphoma with rearrangements of *MYC* and *BCL2* and/or *BCL6* (double hit and triple hit lymphomas). The notion that *MYC* is frequently rearranged in DLBCL-type RS has supported the investigation of R-EPOCH in this disease. R-EPOCH results in 20% response rate in DLBCL-type RS, median PFS of 3 months, and median overall survival of 6 months [52].

Platinum-containing regimens have also been evaluated. The OFAR (oxaliplatin, fludarabine, ara-C, and rituximab) regimen has shown a response rate of 38–50% (CR 6–20%), though responses are of short duration (mean PFS of 3 months and mean OS of 6–8 months). Severe hematotoxicity occurs in 77–95% of cases, severe infection in 8–17%, and treatment-related mortality in 3–8% (Table 10.2) [53, 54].

Treatments developed for highly aggressive lymphomas are severely toxic in DLBCL-type RS. Hyper-CVAD, a fractioned cyclophosphamide, vincristine, doxorubicin, and dexamethasone regimen, induces a response in 41% (CR 38%) of patients, and translates in a median overall survival of 10 months. Severe hematotoxicity occurs in all cases, producing infective complications in 50% of patients, which in turn results in a treatment-related mortality of 14% (Table 10.2) [55]. Combination of rituximab plus hyper-CVAD alternating with methotrexate and ara-C results in a response rate of 43% (CR 38%), and translates into a median overall survival of 8 months. This combination is highly toxic despite the prophylaxis with granulocyte-macrophage colony stimulating factor (GM-CSF) (severe hematotoxicity in 100%

Table 10.2 Summary of the published induction regimens for Richter syndrome[a]

Reference	Study design	Patients	RS type	Regimen	ORR	CR	PFS/FFS	OS	Neutropenia (grade 3–4)	Thrombocytopenia (grade 3–4)	Infection (grade 3–4)	TRM
Dabaja [55]	Clinical trial	26	DLBCL	Hyper-CVXD	41%	38%	na	10 months	100%	79%	39%	14%
Tsimberidou [57]	Clinical trial	16	DLBCL	FACPGM	6%	6%	1 month	10 months	90%	83%	55%	18%
Tsimberidou [56]	Clinical trial	30	DLBCL	R+hyper-CVXD+GM-CSF/ R+HDM-ara-C+GM-CSF	43%	27%	na	8 months	100%	40%	39%	22%
Tsimberidou [58]	Clinical trial	7	DLBCL	^{90}Y ibritumomab tiuxetan	0%	0%	1 month	na	29%	71%	14%	0%
Tsimberidou [53]	Clinical trial	35	DLBCL	OFAR1	50%	20%	3 months	8 months	85%	95%	8%	3%
Tsimberidou [54]	Clinical trial	31	DLBCL	OFAR2	38%	6%	3 months	6 months	89%	77%	17%	8%
Langerbeins [50]	Clinical trial	15	DLBCL	R-CHOP	67%	7%	10 months	21 months	55%	65%	28%	3%
Eyre [51]	Clinical trial	37	DLBCL	CHOP-O	46%	27%	6 months	11 months	33%	25%	51%	0%
Kuruvilla [60]	Clinical trial	6	DLBCL	Selinexor	33%	0%	na	na	na	na	na	na
Hillmen [62]	Clinical trial	29	DLBCL	Acalabrutinib	38%	14%	3 months	na	10%	na	na	na
Tsang [61]	Retrospective	4	DLBCL	Ibrutinib	75%	25%	na	na	na	na	na	na
Ding [64]	Clinical trial	9	DLBCL	Pembrolizumab	44%	11%	na	na	na	na	na	na
Davids [63]	Clinical trial	7	DLBCL	Venetoclax	43%	0%	na	na	na	na	na	na
Bockorny [67]	Retrospective	67	HL	ABVD (31%), MOPP (16%), CHOP (13%), other (40%)	52%	27%	12 months	20 months	na	na	na	na

[a]*RS* Richter syndrome, *ORR* overall response rate, *CR* complete response rate, *PFS* progression-free survival, *FFS* failure free survival, *OS* overall survival, *TRM* treatment-related mortality, *DLBCL* diffuse large B-cell lymphoma, *HL* Hodgkin lymphoma, *hyper-CVXD* fractionated cyclophosphamide, vincristine, liposomal daunorubicin, and dexamethasone, *R+hyper-CVXD+GM-CSF/R+HDM-ara-C+GM-CSF* rituximab, fractionated cyclophosphamide, vincristine, liposomal daunorubicin, dexamethasone and granulocyte-macrophage-colony stimulating factor alternating with rituximab, methotrexate, ara-C and granulocyte-macrophage-colony stimulating factor, *FACPGM* fludarabine, ara-C, cyclophosphamide, cisplatin, and granulocyte-macrophage-colony stimulating factor, *OFAR* oxaliplatin, fludarabine, ara-C, and rituximab, *R-CHOP* rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone, *ABVD* adriamycin, bleomycin, vinblastine, and dacarbazine, *MOPP* mechloretamine, vincristine, procarbazine, and prednisone, *CHOP* cyclophosphamide, doxorubicin, vincristine, and prednisone, *CHOP-O* cyclophosphamide, doxorubicin, vincristine, prednisone plus ofatumumab, *na* not available

of cases, severe infections in 39%, treatment-related mortality of 22%) [56]. The combination of fludarabine, ara-C, cyclophosphamide, cisplatin, and GM-CSF (FACPGM) has limited activity in DLBCL-type RS (response rate of 5%) but significantly toxicity (severe hematotoxicity in 90% cases, infection rate of 55%, treatment-related mortality of 18%) [57]. Though ^{90}Y ibritumomab tiuxetan is active in transformed follicular lymphoma, no responses have been documented in DLBCL-type RS patients treated with radio-immunotherapy (Table 10.2) [58].

Based on these results, and despite the limited level of evidence imposed by small sample size and phase I–II design of trials, R-CHOP is widely used as first-line option for the treatment of DLBCL-type RS.

Stem Cell Transplantation Since the response duration with chemotherapy alone is short, both autologous and allogeneic stem cell transplantation (SCT) have been proposed as post-remission therapies in DLBCL-type RS. Nevertheless, most patients (85–90%) with DLBCL-type RS are unfit or do not achieve adequate response to proceed to transplant.

The European Group for Blood and Marrow Transplantation (EBMT) has retrospectively investigated the role of both autologous and allogeneic SCT as post-remission therapy in DLBCL-type RS (Table 10.3) [59]. By this analysis, the outcome of patients who undergo allogeneic or autologous SCT is encouraging. At 3 years, relapse free survival is 27% after allogeneic SCT and 45% after autologous SCT. The non-relapse mortality at 3 years is 26% after allogeneic SCT and 12% after autologous SCT. Survival at 3 years is 36% after allogeneic SCT and 59% after autologous SCT [59].

SCT could be effective in DLBCL-type RS by two different therapeutic mechanisms: dose intensity delivered by high-dose cytotoxic therapy and, in the case of allogeneic SCT, graft-versus-leukemia activity. An argument in favor of the high-dose principle in DLBCL-type RS is the efficacy of autologous SCT. Although there is no clear *plateau* in relapse-free survival among patients who undergo autologous SCT, only a fraction of relapses is related to RS, while the remaining progressions are due to CLL, suggesting that autologous SCT may eradicate the RS component in many patients even though the underlying CLL may persist. The existence of a graft-versus-leukemia effect in RS might be suggested by the *plateaus* of the relapse free survival among RS patients treated with reduced intensity conditioning allogeneic SCT [59].

Disease activity at SCT is the main factor influencing the post-transplant outcome. Indeed, patients who undergo SCT with a chemotherapy-sensitive disease have a superior survival compared to those who undergo transplantation with active and progressive disease. The major benefit of SCT is obtained in young (<60 years) patients. Among patients receiving allogeneic SCT, those conditioned with a reduced intensity regimen have the longest survival [59]. Overall, these data suggest that both autologous SCT and reduced intensity conditioning allogeneic SCT can be effective in young patients with transformed CLL as long as a status of chemosensitivity is maintained.

Novel Agents Three aspects are in strong support for the development of novel targeted agents in the field of RS: (1) the unsatisfactory response rates obtained with conventional chemo-immunotherapy; (2) the low number of cases that can proceed to transplant because of the constrains imposed by a combination of age, poor performance status, lack donor availability, and refractoriness to induction treatments; and (3) the increased understanding of the cellular programs that are molecularly deregulated in RS. Though phase I/II studies of novel agents show promising signals of single-agent activity in DLBCL-type RS, these results warrant further investigations.

Selinexor is a selective inhibitor of nuclear export. Deregulation of the nucleo-cytoplasmic transport of proteins plays an important role in cancer and depends on the activity of export proteins, including XPO1. XPO1 is the nuclear exporter of several tumor suppressor proteins, including TP53. Tumor cells enhance the export

Table 10.3 Stem cell transplant in Richter syndrome[a]

Reference	Patients	Age < 60years (%)	Transplant	CR/PR at transplant	RIC	VUD	3-year relapse	3-year RFS	NRM	3-year OS	Prognostic factors
Tsimberidou [21, 66]	17	52	Allogeneic	41%	Na	52%	na	na	na	75% (if remission at transplant)	Remission at transplant
Cwynarski [59]	25	60	Allogeneic	60%	72%	44%	47%	27%	26%	36%	• Remission at transplant • Age < 60 years • RIC
Cwynarski [59]	34	65	Autologous	82%	–	–	43%	45%	12%	59%	None

[a]*CR* complete response, *PR* partial response, *RIC* reduced intensity conditioning, *VUD* volunteer unrelated donor, *RFS* relapse free survival, *NRM* non-relapse mortality, *OS* overall survival

of these proteins out of the nucleus, therefore inhibiting their tumor suppressor activity. This notion provides the rationale for blocking XPO1 to retain tumor suppressor proteins in the nucleus and activate them in tumor cells. In a phase I study, selinexor showed signal of activity in 33% of DLBCL-type RS patients that were refractory to the previous chemotherapy regimen (Table 10.2) [60]. Despite this signal, the SIRRT phase 2 study (NCT02138786), which was tailored at establishing the activity of selinexor in relapsed and/or refractory RS patients, has been prematurely terminated due to enrollment challenges and moderate activity in this rare disease.

CLL is addicted to BTK signaling through the BCR, and a proportion of RS shows biased usage of immunoglobulin gene rearrangements suggesting that BCR played a role at a certain timepoint of the transformed disease. Transient activity of ibrutinib has been reported in DLBCL-type RS, including response in three out of four patients (one CR, two PRs). In these patients, the median duration of response was of 6 months (Table 10.2) [61]. Acalabrutinib is a highly selective BTK inhibitor having minimal off-target activity in pre-clinical studies. In the ACE-CL-001 phase I/II trial (NCT02029443) the overall response rate to acalabrutinib among DLBCL-type RS (including relapsed and refractory cases) was 38%, the median progression-free survival 3 months, and the median duration of response 5 months (Table 10.2) [62].

The fact that most of the DLBCL-type RS have *TP53* disruption means that novel drugs for this condition need to act independently of *TP53*. Venetoclax is a specific inhibitor of BCL2 that acts in a *TP53* independent way and is effective in high risk CLL. In the M12-175 (NCT01328626) phase I study, a limited number (n = 7) of DLBCL-type RS were treated with escalating doses of venetoclax, achieving a response rate of 43% (no CRs) (Table 10.2) [63].

DLBCL-type RS frequently occurs upon an exhausted immune system. T-cell exhaustion in CLL is driven, at least in part, by immune checkpoint deregulation, including expression of high levels of checkpoint inhibitory molecules, such as PD-1, on T-cells, and expression of ligands for these molecules, including PD-L1 and PD-L2, on CLL cells. Pembrolizumab, an antibody that targets the PD-1 receptor, provides signals of activity in DLBCL-type RS, including response in four out of nine patients (MC1485 phase 2 trial; NCT02332980) (Table 10.2) [64].

10.7 Suggested Management of DLBCL-Type Richter Syndrome

Based on the available data, mostly derived from retrospective studies, it is difficult to propose a standard and optimized approach for DLBCL-type RS patients. However, some suggestions can be made (Fig. 10.4): (1) adopt a biopsy policy for high risk CLL patients (i.e., those harboring *TP53, NOTCH1* mutations); (2) in the event of a clinical suspicion of transformation, a ^{18}FDG PET/CT should be performed and the open biopsy tailored to the index lesion according to its results; (3) if the biopsy reveals an aggressive lymphoma, the clonal relationship with CLL should be assessed, though it may not be always feasible because of the lack of material of the CLL phase, or because of the formalin fixation of the RS biopsy, which might render the material inadequate for molecular studies; (4) if the CLL and DLBCL are clonally unrelated, treat the disease as a de novo DLBCL; (5) if the CLL and DLBCL are clonally related, consider the patient for a clinical trial; if it is not available, the following approach can be considered: (a) treat with R-CHOP; (b) consolidate young and fit patients with reduced intensity conditioned allogeneic or autologous stem cell transplant depending on whether a donor is available.

10.8 Prognosis of DLBCL-Type Richter Syndrome

The prognosis of DLBCL-type RS is generally poor. A validated RS prognostic score based on five adverse risk factors (Zubrod performance status >1, elevated LDH levels, platelet count <100 × 10^9/L, tumor size >5 cm, and

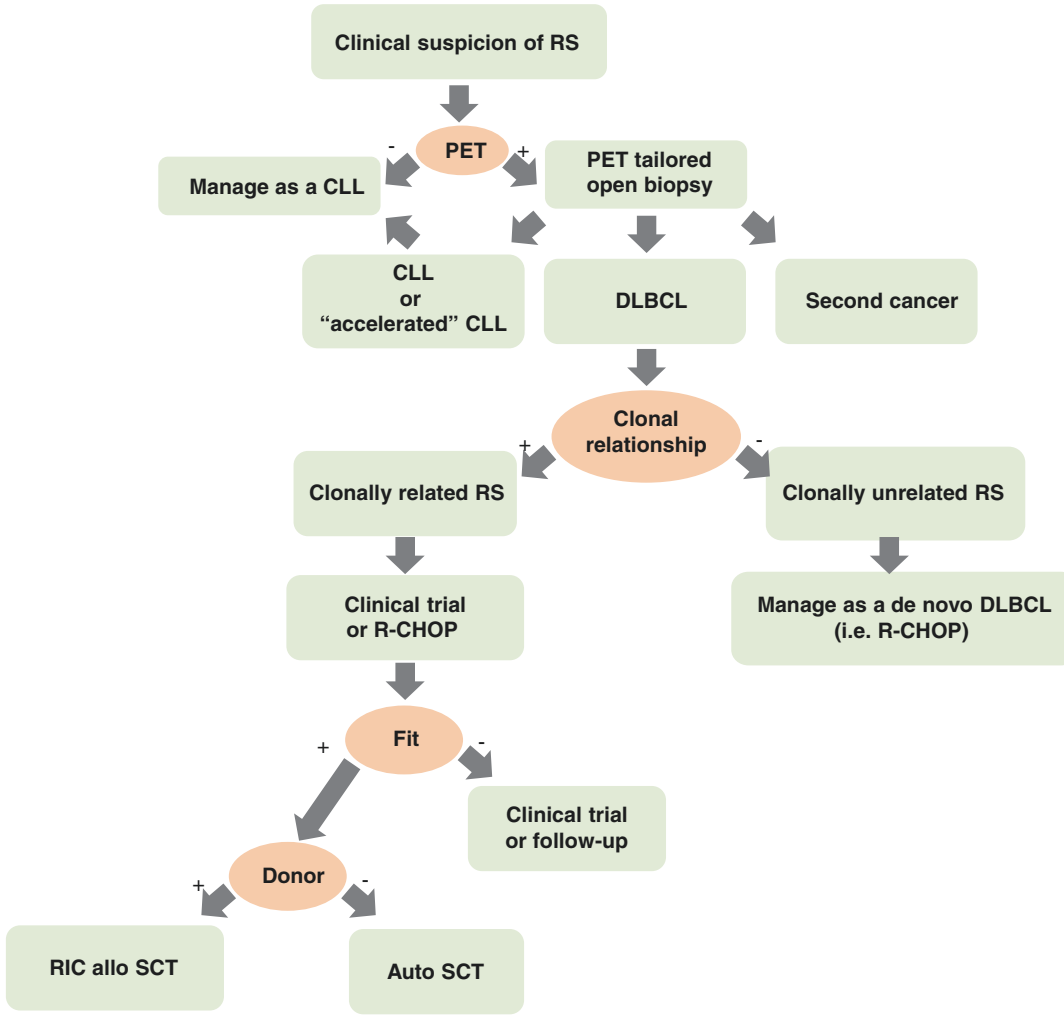

Fig. 10.4 Algorithm for the management of DLBCL-type RS

more than two prior lines of therapy) stratifies four risk groups based on number of presenting risk factors: 0 or 1, low risk (median survival: 13–45 months); 2, low-intermediate risk (median survival: 11–32 months); 3, high-intermediate risk (median survival: 4 months); 4 or 5, high risk (median survival: 1–4 months) [21].

Clonal relationship between CLL and DLBCL clones is the most important prognostic factor, with a longer mean survival (~5 years) for patients with clonally unrelated DLBCL compared with clonally related DLBCL transformation (8–16 months) [4]. As a consequence, investigating the clonal relationship in DLBCL-type RS patients is clinically relevant, especially considering that clonally unrelated DLBCL may be managed as a de novo DLBCL arising in the context of CLL, rather than a true transformation.

10.9 Hodgkin Lymphoma-Type Richter Syndrome

HL-type RS is a rare disease. Indeed, according to the Mayo Clinic CLL database, the 5-year and 10-year incidence of HL-type RS are 0.25% and 0.5%, respectively [65]. No risk factors were found to be relevant in developing HL. Given the

disease rarity, clinical trials have never been performed to assess the treatment of HL-type RS and all the information come from retrospective analyses of single institution or multicentric series. Doxorubicin, bleomycin, vinblastine, dacarbazine (ABVD) is the standard of care for *de novo* HL, and it is the most frequently used regimen for treating HL-type RS. Among HL-type RS treated with ABVD, the response rate ranges from 40% to 60% and the median overall survival is 4 years [65–68]. Though the outcome of HL-type RS is significantly shorter than that of *de novo* HL, it appears to be longer than that observed in the DLBCL-type RS. Therefore, stem cell transplantation is less used for consolidation of HL-type RS.

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Part VII

Related Entities

Prolymphocytic Leukaemia

11

Claire Dearden

11.1 Introduction

Prolymphocytic leukaemia (PLL) was originally described as a rare variant of chronic lymphocytic leukaemia (CLL) characterised by splenomegaly, high white blood cell count, distinct morphology, and a more aggressive disease course. By 1973 it was recognised that there were B-cell and T-cell subtypes of PLL [1]. Although there are similarities in the clinical presentation, these two subtypes can now be readily distinguished from each other and from other mature B- and T-cell leukaemias by their unique phenotypic, cytogenetic, and molecular features [2].

Despite the introduction of novel and more effective therapies, PLL remains incurable. The poor outcome is partly explained by the presence of complex and unfavourable molecular abnormalities. Current therapy relies on the use of monoclonal antibodies with or without purine analogue-based chemotherapy, in some cases consolidated with a haemopoietic stem-cell transplant (SCT). The improved understanding of the pathogenesis and the discovery of specific dysregulated pathways may lead to the development of a more targeted treatment approach in the future.

Given the significant differences between the two subtypes of PLL these will be discussed separately.

11.2 B-PLL

B-PLL is extremely rare, accounting for less than 1% of lymphoid leukaemias. The median age is 69 years and is more commonly seen in males, with a M;F ratio of 1.6:1 [3]. There are no clear genetic or environmental predisposing factors.

11.2.1 Clinical Features

Patients may be asymptomatic at diagnosis with a persistent 'low-grade indolent' phase which may persist for a few years. More typically patients present with a short history characterised by B symptoms, splenomegaly, which is seen in two-thirds of patients and is often massive, and marked lymphocytosis. Lymphadenopathy, although present in more than half of patients, is rarely bulky. CNS involvement is rare.

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11.2.2 Laboratory Diagnosis

Accurate diagnosis is dependent on full integration of laboratory results including peripheral blood morphology, immunophenotyping, cytogenetics, and molecular genetics (Table 11.1). Given the rarity of this leukaemia it is important to ensure that there is input from an experienced specialist haematologist/haemato-pathologist in the interpretation of these tests.

11.2.2.1 Morphology

Prolymphocytes are medium-sized lymphoid cells with basophilic cytoplasm and prominent nucleoli (Fig. 11.1). B-prolymphocytes are usually larger than normal lymphocytes or those found in CLL. By definition they need to comprise more than 55% of circulating lymphocytes [2]. Histology of other tissues such as bone marrow, lymph nodes, and spleen may be helpful in supporting the diagnosis but the key information is usually obtained from extensive analysis of peripheral blood lymphocytes.

Table 11.1 Integrated diagnosis of PLL showing characteristic immunophenotype, cytogenetics, and molecular abnormalities for B- and T-cell subtypes

	B-PLL	T-PLL
Immunophenotype	CD19+, CD20+, SmIG strong, CD5 usually negative, CD23-, SOX11-, cyclin D1—FMC7+, CD 79a+	CD2+, CD3+, CD5+, CD7 strong +, CD4/8 variable, CD52 strong+, TdT-, CD25-
Cytogenetics	t(8,14), del17 no t(11,14)	Complex Inv 14, t(14,14), t(x,14), Iso 8q, 8+, deletions 11q-, 22q-, 13q-,6q-12p-17p-gains 22q, 6p
Molecular abnormalities	*C-MYC, TP53*	*TCL1, MTCP1, ATM, JAK3, STAT5b, IL2RG, EZH2, CHEK2, CDKN1B*

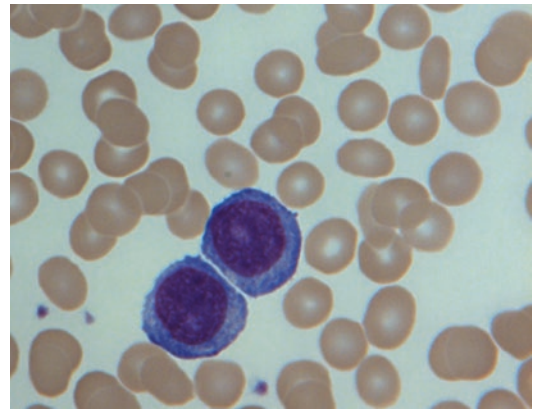

Fig. 11.1 Peripheral blood morphology of B-PLL showing monomorphic prolymphocytes (PL) with condensed chromatin, prominent nucleolus, and scanty basophilic cytoplasm

11.2.2.2 Immunophenotyping

Distinguishing between the B- and T-cell subtypes of PLL is readily achieved through immunophenotyping. It can be more challenging to discriminate between PLL and other T- or B-cell lymphoproliferative disorders. B-prolymphocytes show a light-chain restricted clonal population of mature B-cells. The immunophenotypic profile overlaps with other B-cell lymphomas which characteristically present with splenomegaly and lymphocytosis, such as mantle cell lymphoma (MCL), splenic marginal zone lymphoma (SMZL), and hairy cell leukaemia variant (HCL-v), but is usually distinguishable from CLL [3]. Although a proportion of CLL cases (CLL-PL) may have an increased number of circulating prolymphocytes (less than 55%), the characteristic immunophenotype of CLL is retained in these cells and is different from that of de novo B-PLL.

11.2.2.3 Molecular Genetics

B-cell clonality can be confirmed by immunoglobulin gene rearrangement. The most consistent genetic changes seen in B-PLL are abnormalities of *TP53* (deletion and/or mutation), seen in 50% of cases [4], and abnormalities of *MYC* in over 50% of cases [5, 6]. These aberrations in *MYC* are not necessarily associated with aggressive clinical behaviour, and Ki67 expression is often low. Increased *MYC*-rearrangements, e.g. t(8;14), and

increased *MYC*-copy number have been identified in a high proportion of the small number of cases studied (in one report 5 out of 6 cases). In some cases abnormalities of *C-MYC* and *TP53* are seen together. Gene expression profiling has shown a clear distinction between B-PLL, CLL (including CLL/PL), and SMZL, but a variable overlap with MCL, particularly in those cases with leukaemic presentation [7, 8]. Conventionally, the demonstration of t(11;14) and expression of cyclin D1 and/or SOX-11 separates MCL from B-PLL, but van der Velden et al. have suggested that B-PLL represents a subset of MCL, irrespective of the presence or absence of t(11;14) [8]. There appears to be a spectrum of B-cell disorders presenting with splenomegaly and lymphocytosis and overlapping morphological, immunophenotypic, and genetic features. Where B-PLL sits within this group of disorders, which include MCL, SMZL, and HCL-v, is less clear and the issue has not yet been fully resolved by next generation sequencing.

11.2.3 Treatment of B-PLL

The rarity of PLL means that there is very little published data regarding treatment. In B-PLL there have been a few case reports and small series (<10 patients), but no prospective clinical trials. There is no treatment specifically licensed for this indication. Recommendations here are thus based on best available data and personal experience [9].

11.2.3.1 Watch and Wait

In those patients presenting with an indolent prephase, watchful monitoring is a reasonable approach. This situation may persist for a number of years without any clear evidence that early treatment intervention will be beneficial.

11.2.3.2 First-Line Therapy for B-PLL

The treatment approach has largely been with regimens developed in commoner B-cell disorders, such as CLL. However, it is recognised that B-PLL does appear to have a poorer survival outcome than CLL, with historical reports quoting a median overall survival of 3 years. For this reason, suitable patients may be considered for early allogeneic SCT. The frequent presence of deletions and/or mutations of *TP53* explains in part the poor outcome with conventional chemotherapy. For the 50% of cases with normal *TP53*, however, a conventional chemo-immunotherapy approach with FCR (fludarabine, cyclophosphamide, rituximab) or BR (bendamustine, rituximab) is reasonable [10–12]. In these reports, an anthracycline (mitoxantrone or epirubicin) has been added, but it is unclear to what extent this improves outcome. Randomised trials in CLL have previously failed to demonstrate superior response rates or improved progression-free survival (PFS) with the addition of an anthracycline and this certainly adds to toxicity. For those patients with non-functional *TP53,* alemtuzumab has historically been the mainstay of treatment and, although no longer licensed in CLL, is available via a patient access scheme [13].

More recently, in CLL, the B-cell receptor (BCR) inhibitors (ibrutinib and idelalisib) have been shown to have activity in 17p-deleted CLL, with similar outcomes when compared to those patients with no deletion [14, 15]. This has led to the licensing of both these agents for first-line therapy in 17p-deleted CLL. B-PLL has been excluded from most of the trials on CLL. Therefore, in the absence of any specific prospective clinical trial data for B-PLL, it would seem that BCR inhibitors may be an effective therapeutic option, especially in 17p-deleted cases, and experience with these and other novel agents is likely to emerge over the next few years. Eyre et al. have reported the results of 5 patients with B-PLL and *TP53* disruption (deletion and/or mutation) who were successfully treated with the combination of idelalisib and rituximab using the same treatment schedule as in CLL [16]. Three patients had received prior therapy (high dose steroids, BR, and alemtuzumab) and 2 were treatment naïve. All patients responded. One patient died of an unrelated lung cancer and treatment was ongoing (>6 months) in the remaining four at the time of the report. Toxicities were largely predictable and manageable and included one case of CMV reactivation.

11.2.3.3 Treatment of Relapsed or Refractory B-PLL

In B-PLL, depending on the remission duration following first-line treatment, relapse can be managed with the same or similar chemo-immunotherapy regimens. Patients with early relapse or with relapse associated with high-risk genetics (e.g. abnormal *TP53*) may be considered for treatment with novel BCR inhibitors such as ibrutinib or idelalisib, or other experimental therapies, preferably within a clinical trial setting. The BCL-2 inhibitor venetoclax has recently been licensed for the treatment of relapsed/refractory and *TP53* deleted CLL. There is no published experience in B-PLL to date but it would be reasonable to assume that this agent may also have a role in the treatment of this disorder [17].

11.3 T-PLL

T-PLL accounts for 2% of mature lymphocytic leukaemias in adults and is the commonest of the T-cell leukaemias [2]. The median age at presentation is 61 years of age and there is a male predominance, with a M;F ratio of 2:1 [18]. Three cases of children with T-PLL have been reported, although incomplete diagnostics were reported in one [19–21]. Patients with ataxia telangiectasia (AT) are at increased risk of developing T-PLL (as well as other lymphoid malignancies) with a younger median age at presentation of approximately 31 years of age [22]. It appears to be a rare complication, in one study only 3 out of 279 patients with AT developed T-PLL [22]. An individual with Nijmegen breakage syndrome developing T-PLL has been reported [23]. Aside from these findings no other genetic or environmental risk factor has been robustly identified thus far.

11.3.1 Clinical Features

Most patients with T-PLL present with a brief history of B symptoms, hepatosplenomegaly, and a marked lymphocytosis (typically >100 × 10^9/l) [18]. Lymphadenopathy, although present in a majority of patients, is rarely bulky. Anaemia and thrombocytopenia are seen in up to half of patients. Erythematous or nodular skin rashes involving the trunks or limbs, peripheral oedema, and pleuroperitoneal effusions may be seen in up to a quarter of patients with T-PLL. T-PLL may also involve the face, where it manifests as purpura and oedema, often in a periorbital distribution [24, 25]. Central nervous system (CNS) involvement is rare. A minority of patients have no symptoms at diagnosis. This 'indolent' phase can persist for a variable length of time and can be as long as years [26]. Disease progression may be rapid when it occurs.

11.3.2 Laboratory Diagnosis

As with B-PLL the diagnosis of T-PLL relies on an integrated evaluation of peripheral blood and bone marrow morphology, immunophenotyping, cytogenetics, and molecular tests (Table 11.1).

11.3.2.1 Morphology

The 'typical' morphology observed in the majority (75%) of T-PLL cases consists of medium-sized lymphoid cells with partial chromatin condensation, a visible nucleolus, and a round or oval nucleus (Fig. 11.2) [2, 18]. A slight basophilic cytoplasm is present, often with protrusions or 'blebs' and an absence of granules. A 'small cell variant' is seen in 20% of cases. These small cells possess condensed chromatin

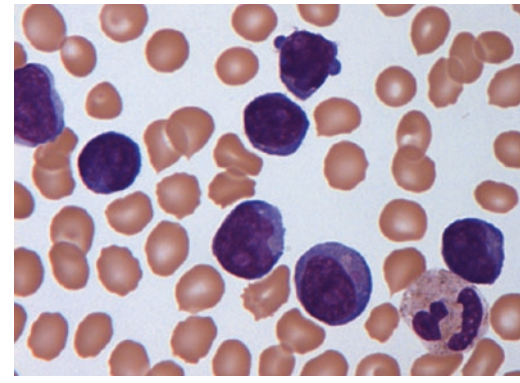

Fig. 11.2 Peripheral blood morphology of T-PLL showing medium-sized lymphoid cells with a regular nuclear outline, single nucleolus, and intense basophilic cytoplasm. An occasional cell shows a cytoplasmic protrusion

with a small nucleolus often difficult to visualise under light microscopy. Finally, the 'cerebriform (Sézary cell-like) variant' is seen in 5% of patients in which the morphology resembles the Sézary cells seen in Sézary syndrome (SS)/mycosis fungoides. The bone marrow is infiltrated in an interstitial pattern by cells with a similar morphology to that seen in the peripheral blood. Skin biopsy of affected areas demonstrates a wide cytomorphological spectrum similar to that observed in the peripheral blood, with a perivascular or diffuse dermal infiltrate, sometimes with accompanying haemorrhage [24, 25]. This is distinct from the typical histology of MF which shows epidermotropism and Pautrier's abscesses. The spleen demonstrates an atrophied white pulp with dense lymphoid infiltrates in the red pulp that invade the capsule. Lymph node involvement is diffuse, often with prominent high-endothelial venules. Without the knowledge of the peripheral blood morphology and immunophenotype the appearance in tissues can be misinterpreted as a peripheral T-cell lymphoma, usually PTCL-NOS.

11.3.2.2 Immunophenotype

Flow cytometry confirms a post-thymic T-cell population (TDT-, CD1A-, CD5+, CD2+, and CD7+) [2]. The majority of cases are CD4+/CD8-. Dual CD4+/CD8+ cells occur in approximately 25% of cases (this is unique to the post-thymic T-cell malignancies) and only a minority of cases express a CD4-/CD8+ phenotype. CD52 is expressed strongly. Cytoplasmic CD3 is always present, but membrane expression may be weak or negative. Natural killer (NK) cell and cytoplasmic granule markers are consistently negative. Typically CD7 expression is strong, whilst CD25 may be negative, thus helping to distinguish T-PLL from adult T-cell leukaemia (ATL) and SS. T-PLL patients are also negative for human T-cell leukaemia virus type 1 (HTLV-1).

11.3.2.3 Molecular Genetics

T-cell receptor genes are rearranged and are identical, confirming a clonal expansion of T-cells. Although cytogenetic and mutational analysis does not alter management, the identification of abnormalities can aid diagnosis as well as provide insight into the pathogenesis of T-PLL.

The most frequently observed group of cytogenetic abnormalities involves chromosome 14 (90%). These may take the form of inv(14), t(14;14)(q11;q32) which involves the *TCL1A* and *TCL1B* locus and t(X;14)(q28;q11) involving a homologue of *TCL1*, *MTCP1* (mature T-cell proliferation 1 gene), which is located on the X-chromosome [27]. Transgenic mouse models have confirmed the oncogenic roles of TCL1 and MTCP1 [28, 29], and functional work identifies TCL1 as an Akt kinase co-activator, promoting cell survival and proliferation [30]. Cytogenetic abnormalities involving chromosome 8 are the next most frequently observed (idic(8p11), t(8;8), and trisomy 8q), in over 50% of cases [31, 32]. Other recurrent abnormalities seen with conventional techniques include loss of 11q23 (ATM inactivation) together with additional losses (22q, 13q, 6q, 9p, 12p, and 17p) and gains (22q and 6p) [31, 32]. Deletion of 12p13, which probably occurs in up to half of T-PLL cases, is thought to contribute to the pathogenesis of T-PLL by causing haplo-insufficiency of *CDKN1B*, which encodes for a protein essential in cell cycle regulation [33]. With the advent of high-throughput sequencing, further driver mutations in T-PLL have been identified [34–37]. These include highly recurrent, largely exclusive, gain-of-function mutations involving *IL2RG*, *JAK1/3*, and *STAT5B*, which lead to constitutive STAT5 signalling. Deleterious mutations in *EZH2, FBXW10,* and *CHEK2* may further contribute to the pathogenesis of T-PLL through their roles in DNA repair, epigenetic transcriptional regulation, and proteasome degradation pathways. It remains unclear how these abnormalities evolve during the course of the disease but it seems likely that, given the high frequency, changes in *TCL1* and *ATM* are early events promoting cell proliferation and survival with impaired DNA repair mechanisms. Secondary mutations, e.g. in the JAK/STAT pathway and those in epigenetic regulators, occur subsequently and lead to further activation of cellular pathways. Additional genomic analysis including sequential tumour sequencing may better define driver mutations and improve the understanding of the clonal

architecture and evolution of T-PLL. Understanding the functional consequence of these mutations is essential in furthering our knowledge of T-PLL and developing novel therapeutics.

11.3.3 Treatment of T-PLL

As with B-PLL, little published data exists regarding treatment of T-PLL and no randomised clinical trials have been conducted. The following recommendations are based on best available evidence from a relatively few small clinical studies [9].

11.3.3.1 Watch and Wait

Not all patients diagnosed with T-PLL require treatment immediately. Chemo-immunotherapy can be associated with significant toxicity and, apart from SCT, current therapy in T-PLL is not curative. Furthermore, some patients present with an 'indolent phase' of the disease [26]. Although disease progression eventually occurs, patients can be monitored for years before requiring intervention. Close monitoring (for example, blood count and clinical examination at regular intervals) is required as disease progression can be rapid and fatal. A pre-treatment lymphocyte doubling time (LDT) of less than 8.5 months has been shown to be associated with a worse outcome [38]. Indications for treatment include B symptoms, symptomatic anaemia or thrombocytopenia, disease infiltration in the skin, lungs, or CNS, and progressive disease demonstrated by an increasing lymphocytosis or rapidly enlarging spleen, liver, or lymph nodes.

11.3.3.2 First-Line Therapy for T-PLL

Treatment is initiated with the aim of attaining a complete response (CR) and patients should be offered a clinical trial when available. There is a limited response to conventional treatment regimens such as alkylating agents or anthracyclines, with a median overall survival (OS) of 7 months in historical series [18]. In the absence of a clinical trial, patients should be offered alemtuzumab (anti-CD52). This was initially employed as monotherapy over 2 decades ago and was first used due to the strong CD52 expression on treatment-naïve T-PLL cells. Studies have shown an overall response rate (ORR) of >80% in the first-line setting and in 50–76% of relapsed-refractory cases [39–41]. Intravenous administration of alemtuzumab is more effective than subcutaneous administration; therefore, intravenous therapy with alemtuzumab should always be the preferred way of administration [42]. Although progression-free survival (PFS) is longer when compared to other therapies (over a year in responders), relapse invariably occurs and there are few long-term survivors, with a median overall survival (OS) from treatment of less than 2 years. For this reason, eligible patients should be considered for consolidation therapies such as SCT.

Alemtuzumab is administered intravenously at a dose of 3 mg on day 1; 10 mg on day 2; and 30 mg on day 3, followed by 30 mg doses every Monday, Wednesday, and Friday beginning the following week until maximum response. Infusion-related reactions are common in the first week and pre-medication with hydrocortisone, paracetamol, and piriton is necessary. Pethidine may be useful to control significant rigors. Alemtuzumab increases an individual's susceptibility to opportunistic infections. Patients should therefore be on appropriate antibacterial and antiviral prophylaxis and undergo serology testing for cytomegalovirus (CMV) and hepatitis B and C prior to commencement of treatment. In previously exposed individuals CMV PCR should be monitored weekly during therapy.

The results of alemtuzumab therapy compare favourably with outcomes reported with the use of purine analogues in which ORR is <50% and remission durations are less than 1 year [43–45]. Single-agent pentostatin has shown the greatest efficacy of all purine analogues in T-PLL [43], although no randomised controlled trials have directly compared single-agent pentostatin and alemtuzumab. Small prospective studies have evaluated the use of alemtuzumab in combination with chemotherapy agents. For example, Hopfinger et al. reported a prospective multicentre phase II trial investigating the use of fludarabine, mitoxantrone, and cyclophosphamide

(FMC) induction followed by alemtuzumab in 16 treatment-naïve patients and 9 previously treated patients [46]. The ORR to FMC was 68% increasing to 92% following the addition of alemtuzumab. Median OS and PFS were 17.1 months and 11.9 months, respectively.

11.3.3.3 Therapy for Relapsed and Refractory T-PLL

In patients who relapse, approximately half can achieve a second disease remission with further alemtuzumab therapy. This is usually of shorter duration. Flow cytometry should be repeated, as T-PLL cells can lose CD52 expression. Pentostatin alone has demonstrated efficacy as a single-agent in a small retrospective study of relapsed T-PLL. The ORR was 45% independent of previous treatment with a median PFS and OS of 6 months and 9 months, respectively [43]. A phase II study evaluated the combination of alemtuzumab with pentostatin in 13 patients with T-PLL. The ORR was 69% with a median OS and PFS of 10.2 and 7.8 months, respectively [47]. Patients who fail to achieve a remission with first-line single-agent alemtuzumab may benefit from the addition of pentostatin to the treatment regimen. Other treatment options include nelarabine or bendamustine, although durable remissions with these therapies are uncommon [45, 48]. Herbaux et al. report 15 patients with T-PLL treated with bendamustine, 7 of whom had failed front-line therapy with alemtuzumab. The ORR was 53% (20% CR), median PFS of 5 months, and OS of 8.7 months, independent of prior exposure to alemtuzumab [48]. Treatment of patients with relapsed or refractory disease is currently suboptimal. Effective novel therapies are needed to improve the outcomes for these patients.

11.3.3.4 Novel Therapies for T-PLL

New approaches aim to utilise our expanding knowledge of T-PLL in order to target pathways involved in disease pathogenesis and resistance. Histone-deacetlyase inhibitors (HDACi) in combination with hypo-methylating agents aim to act synergistically to increase expression of silenced tumour suppressor genes. The combination of cladribine and alemtuzumab with or without an HDACi can overcome alemtuzumab resistance and induce expression of other molecules, such as CD30 liable to targeting with additional agents [49]. Cells with inactive ATM demonstrate impaired DNA double strand break repair capabilities. Poly (ADP-ribose) polymerase (PARP) inhibition imposes the requirement for DNA double strand break repair capabilities and therefore selectively sensitises ATM-deficient tumour cells to killing [50]. Chimeric antigen receptor natural killer cells targeting T-cell specific antigens, such as CD7, may represent a novel therapeutic avenue not yet explored in vivo [51]. The difficulty with this approach is the need to retain normal T-cells for effective immunity, since these antigen-specific CAR-T cells eliminate the entire T-cell population (fratricide). The duplication of the TRBC locus can be exploited in this regard since normal T-cell populations will contain a mixture of cells expressing either TRBC1 or TRBC2, whilst malignant populations will express only one type. The malignant cell can thus be targeted with a specific TRBC1 or 2 allowing survival of the alternate normal T-cell population and preserving T-cell immune function.

Given the high frequency of mutations observed, and the perturbed signalling pathways, small molecule inhibitors targeting pathways such as JAK-STAT may represent another therapeutic strategy available for patients [52]. The presence of JAK 3 mutations confers a worse prognosis for patients with T-PLL [53]. It is important to understand the functional consequences of the gene mutations in order to inhibit the activated pathway. Studies targeting a specific mutation or activated pathway have had variable success in inhibiting the growth and/or survival of T-PLL cells ex vivo, suggesting that genetic biomarkers do not always translate into an effective therapeutic strategy. Inhibition of ITK, for example, had very low in vitro efficacy in primary T-PLL cells and cell lines despite the prominent signature of T-cell receptor (TCR) signalling components [54]. High-throughput ex vivo drug sensitivity and resistance testing of T-PLL cells in conjunction with genetic profiling has demonstrated sensitivity to a variety of compounds

including HDAC inhibitors, CDK inhibitors, BCL-2 inhibitors, PI3K/AKT inhibitors, and JAK-STAT inhibition [55]. Of these, HDAC and BCL-2 inhibitors were the most promising. However, with this methodology it is important to mimic the pharmacological and therapeutic effect in vivo. For example, in assay systems cells may be exposed to a drug continuously whilst in vivo the effects may only be present for a few hours depending on the delivery schedule of the drug. A recent report has shown that ex vivo responses to venetoclax, a bcl-2 inhibitor, were confirmed in vivo in two late stage T-PLL patients [56]. Future combination studies with this agent are planned.

11.4 Haematopoietic Stem-Cell Transplant in PLL

In PLL, relapse seems inevitable and remissions are generally of short duration. In addition, B-PLL is often characterised by high-risk genetic abnormalities of *TP53*. For these reasons, it is appropriate to consider potentially curative treatment with allogeneic SCT in first remission for eligible patients (Table 11.2). Unfortunately, the age and fitness of patients with PLL often rules out this approach, although reduced-intensity conditioning (RIC) regimens have widened applicability in recent years. In B-PLL, there are a number of case reports of successful transplants although inevitably case reports are misleading because they fail to highlight the number of unsuccessful cases [57–59]. Kalaycio et al. report on 11 cases of B-PLL with a median follow-up of 13 months [58]. At 1 year, PFS was 33% and TRM was 28%. They saw no difference between reduced-intensity and full myelo-ablative conditioning.

In T-PLL approximately 80% of patients achieve a CR following alemtuzumab treatment. However, without additional therapy, a majority of patients will relapse within 2 years. A number of studies have investigated the use of allogeneic SCT in T-PLL to consolidate remissions which suggest that OS can be improved and for a minority of cases can achieve a cure [60–63]. The main challenges are the treatment-related mortality (TRM) and the risk of relapse. The European Group for Blood and Marrow Transplantation (EBMT) registry had 41 patients with T-PLL who had received an allogeneic SCT [61]. Three-year OS was 21% with TRM and relapse rates of 41 percent (although nearly half of the patients had refractory disease at the time of transplant). A similar TRM rate and lower 3-year relapse rate were reported from a smaller cohort of patients although a larger proportion of patients in our study were in CR, highlighting the importance of disease-status at the time of SCT [60]. A retrospective study by Guillaume et al. reported 27 patients undergoing allogeneic SCT (14 of whom were in CR at the time of SCT) [62]. The relapse rate at 3 years was 47%, with a TRM of 31% and an OS of 36%. Given the increasing use of reduced-intensity conditioning and matched unrelated donors, as well as improvements in supportive care, more patients are eligible for SCT and the current data may not be applicable to prospective cohorts of T-PLL patients.

Table 11.2 Allogeneic stem-cell transplant in PLL

Patient number	Median age in years (range)	Status at transplant	TRM at 3 years	Relapse rate at 3 years	3 year OS	Reference
47 PLL 21 T 11 B 15 NK	54 (30–75)	16 CR, 8 PR, 21 refractory	28% at 1 year	39% at 1 year	1 year OS 48%	Kalaycio [58]
13 T-PLL	51 (39–61)	10 CR, 1 PR	31%	33%	62%	Krishnan [60]
41 T-PLL	51 (24–71)	11 CR, 12 PR	41%	41%	21%	Wiktor-Jedrzejczak [61]
27 T-PLL	54 (36–65)	14 CR	31%	47%	36%	Guillaume [62]
10 T-PLL	59 (43–72)	8 CR, 2 PR	30%	50%	50%	Sellner [64]

TRM transplant related mortality, *OS* overall survival, *CR* complete remission, *PR* partial remission, *NK* not known

Relapse following allogeneic SCT is associated with a dismal outcome. Most commonly relapses occur within the first 3 years, with a peak incidence in year 1. However, late relapses, although rare, can occur. Szuszies et al. reported on 3 T-PLL patients who had undergone a RIC allogeneic SCT who had good initial engraftment but subsequent diminishing donor chimerism, which was restored following donor lymphocyte infusions (DLI) [63]. Further evidence of a graft versus leukaemia (GvL) effect, albeit limited and transient, was provided by a study of 10 T-PLL patients undergoing allo-SCT who had serial measurement of minimal residual disease (MRD) post-transplant. Sellner et al. showed that MRD kinetics could be correlated with relapse and was used in the study to direct immunomodulation (e.g. DLI) [64]. However, only 2 patients achieved durable MRD negativity and the GvL effect appeared to be due to a polyclonal rather than a directed monoclonal T-cell response [64]. Close monitoring and early intervention may be beneficial to pre-empt full blown relapse.

Some patients for whom allogeneic transplant was not suitable have undergone autologous SCT [60]. The TRM was much reduced (6%) but the relapse rate was 87%. However, a median PFS of 18 months and OS of 49 months (vs 20 months) with 37% alive at 5 years (vs 13%) were significantly better than for a group of matched patients who did not undergo any form of consolidation after achieving a durable (>6 months) CR [60]. This remains a treatment option for selected patients.

11.5 Summary

PLL comprises two subtypes, T-cell and B-cell, both of which are rare lymphoid malignancies with aggressive clinical course and poor prognosis. Clinical presentation, with splenomegaly and high lymphocyte count, may be similar but the biology and genetics is quite distinct. Although a subset of patients may have an indolent phase of variable length, progression is inevitable. Treatment is not curative but can deliver high response rates and reasonably durable remissions, measured in years for those achieving CR. For B-PLL first-line therapy is with combination chemo-immunotherapy for patients with normal *TP53* and with alemtuzumab or BCR inhibitors for those with deletions or mutations of *TP53*. For T-PLL first-line therapy is with intravenous alemtuzumab. Allogeneic SCT should be considered for eligible patients. Novel therapies targeting key pathways, JAK-STAT in T-PLL and BCR signalling in B-PLL, are likely to provide new approaches in the future.

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12 Large Granular Lymphocyte Leukemia

Jan Dürig

12.1 Introduction and Disease Definition

The term *large granular lymphocyte (LGL) leukemia* was first coined by Loughran et al. in 1985 and at that time described a rare chronic lymphoproliferative disease of mature T- or natural killer (NK)-cells [1]. The 2008 World Health Organization (WHO) classification of mature T- and natural killer (NK)-cell neoplasms recognized three different subtypes of LGL leukemia (LGL-L), i.e., T-cell large granular lymphocyte leukemia (T-LGL-L), aggressive NK-cell leukemia (AKNL), and the provisional entity chronic lymphoproliferative disorder of NK (CLPD-NK) cells [2]. These categories were adopted by the most recent WHO (2016) classification [3] which further highlights clinical features associated with activating mutations in STAT3 and STAT5B, which can be detected in a subgroup of patients.

Different from aggressive NK-cell leukemia which is characterized by rapid disease progression, chemotherapy resistance, and a dismal prognosis with short overall survival [4], T-LGL-L and CLPD-NK, which share many biological and clinical features, typically follow an indolent clinical course [5, 6]. Disease-related mortality is mainly due to infections occurring in <10% of the patients [7–9], and the median overall survival has been estimated to be 9–10 years [7]. Here, we focus on these two latter disease entities and review recent work related to epidemiology, diagnosis, clinical presentation, pathogenesis, and current treatment concepts.

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12.2 Epidemiology

LGL leukemia is a rare disease which accounts for 2–5% of chronic lymphoproliferative disorders in North America and Europe and 5–6% in Asia. Recently published population-based data from the Dutch registry and the United States Surveillance, Epidemiology, and End Results program (SEER) revealed an incidence of 0.2–0.7 cases per 1,000,000 individuals [7, 8]. Males and females are similarly affected and the median age at diagnosis was 66.5 years [7]. By contrast aggressive NK-LGL is mainly observed in Asia, where it occurs in association with Epstein–Barr virus infection and predominantly affects younger adults [4, 10].

12.3 Diagnostic Workup

LGL leukemia is characterized by the sustained (at least 6 months) presence of an expanded clonal T- or NK-cell population in the peripheral blood. The diagnosis requires a multimodality

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approach integrating evaluation of cell morphology (Fig. 12.1), immunophenotyping, T-cell receptor (TCR) rearrangement, and STAT3 gene sequencing studies (Fig. 12.2) with information regarding the comorbidities and clinical history of the patient [2, 3, 5, 6]. LGL leukemia is often suspected in patients exhibiting increased numbers of LGL (normal range < 0.3×10^9/l) in the peripheral blood. A persistent LGL count of more than 2×10^9/l is compatible with the diagnosis; however, there is no consensus on a specific threshold [5, 6, 11]. The disease may be diagnosed in patients presenting with lower numbers of clonal LGL, if they display other typical clinical or hematological features such as rheumatoid arthritis (RA) or cytopenias. Normally, leukemic LGLs are easily identified on blood smears by their characteristic morphology; however, they cannot be distinguished from reactive cytotoxic lymphocytes. Morphologic hallmarks include a large cell size (15–18 μm), round nuclei with mature chromatin, and an abundant pale-blue cytoplasm with prominent sparse azurophilic granules (Fig. 12.1) [3, 12, 13]. T-LGL-L exhibits considerable morphological heterogeneity (Fig. 12.1) and a significant subgroup of patients (23%) appears to be characterized by the presence of high numbers of LGL with a diameter of 12.5 μm or smaller (Fig. 12.1d) [14]. Intriguingly, this small variant T-LGL-L was associated with younger age, anemia, activating STAT3 mutations, and a more benign clinical course and may thus be considered a new subcategory of T-LGL-L, although this interesting finding awaits confirmation in larger patient series [14].

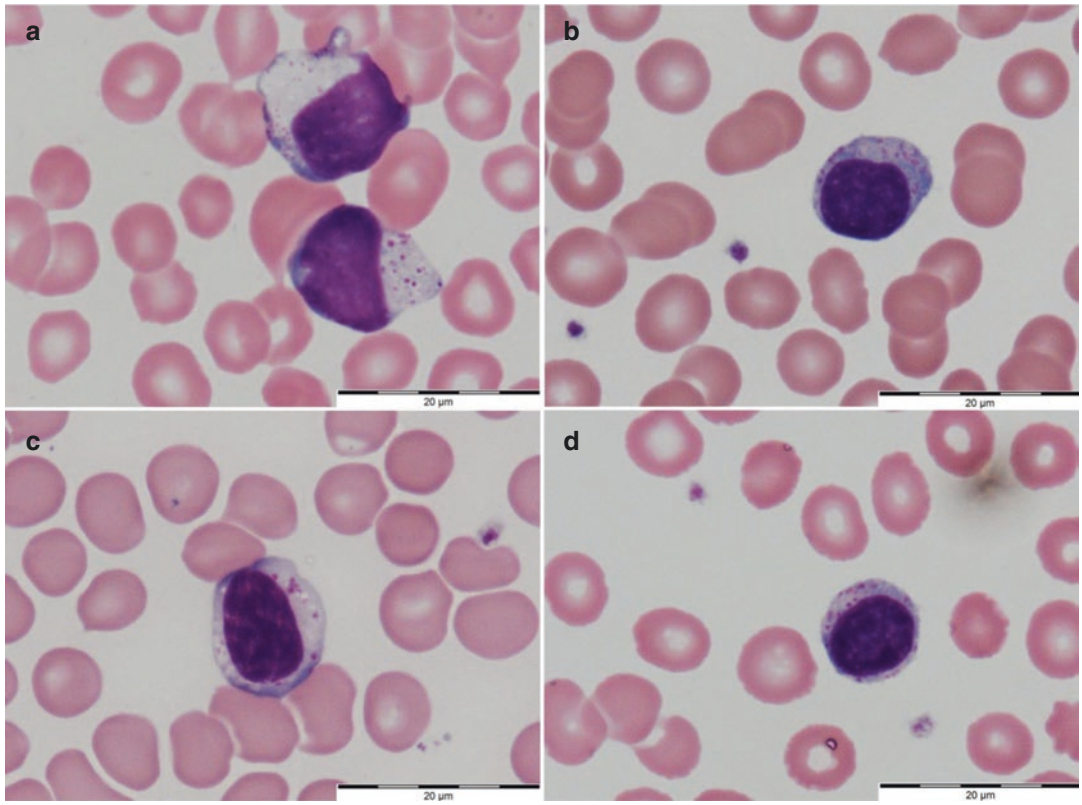


Fig. 12.1 Large granular lymphocytes on blood smears. Panels (**a**)–(**d**) show typical LGLs on blood smears from four individual patients with T-LGL. Note the inter- (**a**–**d**) and intraindividual (**a**) morphologic variability of cytoplasmatic granules and sizes of the leukemic cells. In panel D a representative LGL from a patient with small variant T-LGL [14] is depicted (Wright–Giemsa stain: original magnification ×1000, Olympus BX53, Olympus SC50)

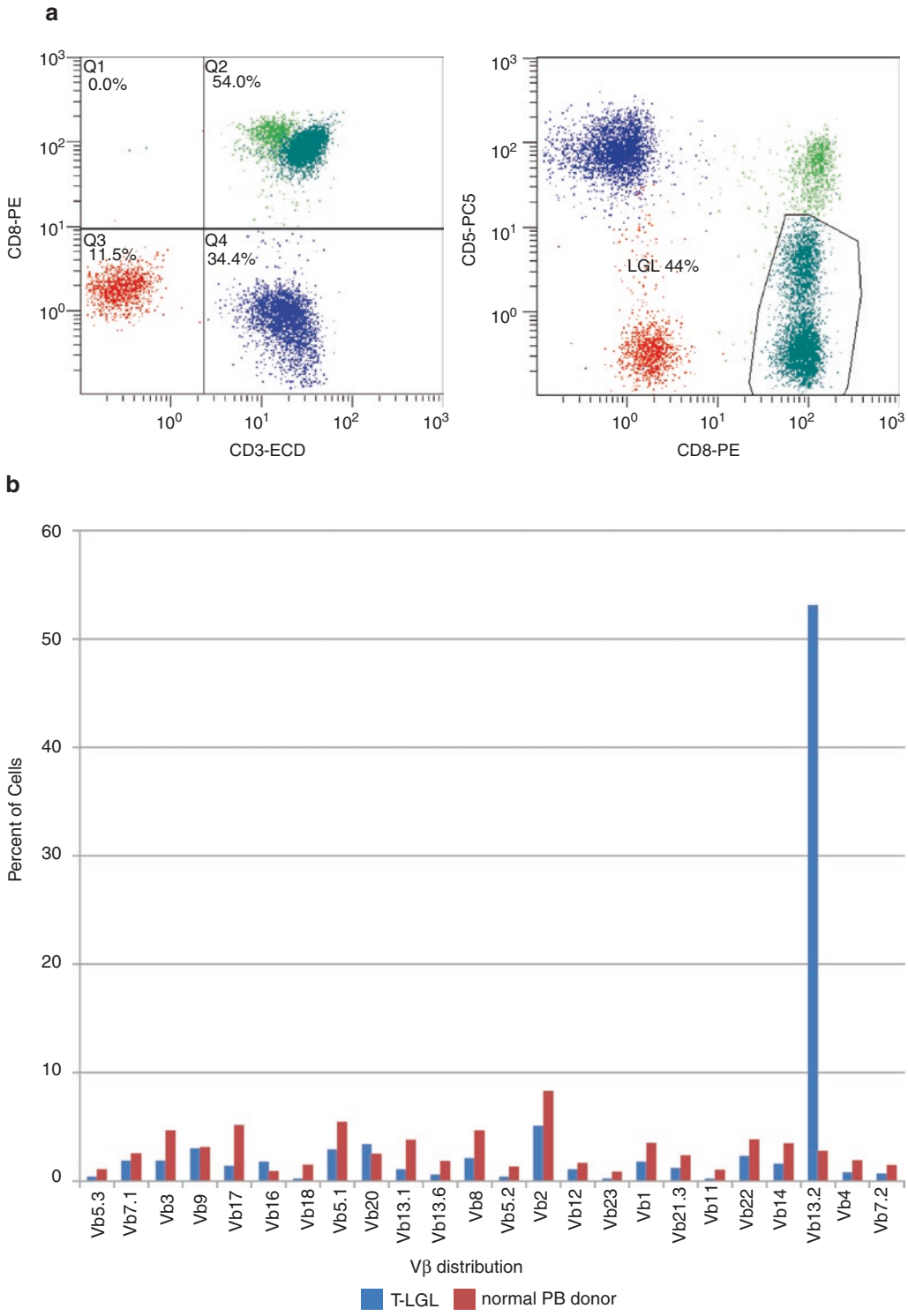
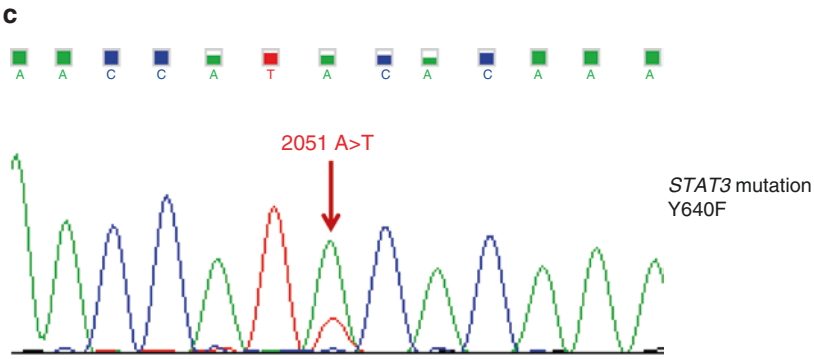


Fig. 12.2 Results of flow cytometry and STAT3 sequencing in a patient with typical T-LGL. (**a**) Flow cytometry analysis of lymphocyte gated peripheral blood cells reveals that the majority of the cells are CD3-positive and CD8-positive (panel on the left). The neoplastic LGL population marked in dark green exhibits characteristic down-regulation of the pan T-cell antigen CD5 (panel on the right). (**b**) Clonality assessment using a panel of Vβ monoclonal antibodies showing predominance of Vβ13.2 T-cells. (**c**) *STAT3* mutation detection using Sanger sequencing

Fig. 12.2 (continued)



Multiparameter flow cytometry is essential in the diagnostic workup of LGL leukemia (Fig. 12.2a). In T-LGL-L it shows a mature post-thymic CD3+, TCRαβ+, CD4-, CD5dim, CD8+, CD16+, CD27-, CD28-, CD45RO-, CD45RA+, and CD57+ constitutively activated T-cell phenotype [3, 15]. Few cases are TCRγδ+ [16], and a rare CD3+CD56+ T-LGL-L subset may be associated with activating STAT5B mutations and exhibit a more aggressive clinical behavior [17]. CLPD-NK does not show surface CD3 but expresses CD8, CD16, CD56, and often abnormal expression of killer immunoglobulin-like receptors (KIRs) [12].

In most of the T-LGL-L cases the neoplastic cells express an abnormal phenotype with frequent down-regulation of surface CD5 or CD7 and aberrant co-expression of the NK-cell markers CD16 and/or CD57 [15]. Flow cytometry can also be used to demonstrate T-cell clonality using a panel of Vß TCR specific monoclonal antibodies, which covers 75% of the Vß spectrum (Fig. 12.2b) [18, 19]. In routine practice these studies are complemented by TCR γ-polymerase chain reaction analyses to ascertain clonality of the disease. Establishing clonality in CLPD-NK is more difficult, because these cells lack TCR expression. Here, a restricted cell surface expression of KIRs may be used as a marker for monoclonal cell expansion [20, 21].

At the molecular level LGL leukemia is characterized by constitutive STAT3 activation [22], which is caused by somatic gain-of-function mutations in 28–75% of T-LGL-L and 30–48% of CLPD-NK cases [23]. The variability regarding the STAT3 mutation frequency in these studies can be explained by differences in patient selection and sequencing techniques, where targeted amplicon sequencing was shown to be more sensitive than conventional Sanger sequencing (Fig. 12.2c) [5, 24, 25]. Mutations are primarily located in exons 20 and 21 encoding for a part of the Src homology 2 domain [23, 25], which plays an important role in the dimerization and activation of the STAT3 protein [25, 26]. These mutations are pathognomonic for LGL leukemias and may also be used as an (additional) marker of clonality in both T-LGL-L and more importantly the CLPD-NK subtype where clonality studies are more difficult. Therefore STAT3 exon 20 and 21 sequencing is now considered essential in the diagnostic workup of patients exhibiting LGL expansions. In a small subset of CD8+ T-LGL (2%) patients activating mutations in the SH2 domain of the STAT5B gene were discovered, which were correlated with an aggressive clinical course of the disease. Interestingly, the mutation frequency of STAT5B was found to be 55% in patients with the rare subtype of CD4+ T-LGL [27]. In contrast to the aggressive disease observed in CD8+ T-LGL with STAT5B mutations, patients suffering from CD4+ T-LGL leukemia exhibited an indolent course independent of their STAT5B mutation status. Because of its high frequency in CD4+ T-LGL, STAT5B mutations have been suggested as a novel diagnostic marker in this rare specific disease subtype [27].

Similar to CLL, in most cases LGL leukemia can be readily diagnosed using peripheral blood and therefore bone marrow aspiration/biopsy is not routinely recommended in the course of initial evaluation [5]. However, if the analysis of

peripheral blood is not diagnostic, bone marrow studies may provide valuable additional information. On immunohistochemistry interstitial clusters of CD8, T-cell-restricted intracellular antigen (TIA)-1 and granzyme B-positive lymphocytes are quite specific of bone marrow involvement by T-LGL-L [28, 29]. Also marrow studies may allow for the assessment of other hematological diseases known to be associated with T-LGL-L such as myelodysplastic syndrome (MDS), aplastic anemia, or B-cell lymphomas. Of note, different from the situation in CLL the percentage of neoplastic LGL in the bone marrow does not correlate with the degree of cytopenia(s) observed in the peripheral blood [30]. This finding suggests that LGL-induced suppression of hematopoiesis may be due to paracrine or direct cell-contact mediated effects rather than physical replacement of healthy bone marrow cells by the leukemic cell clone.

12.4 Clinical and Laboratory Features

The clinical presentation of LGL leukemia is dominated by neutropenia with recurrent infections, anemia, splenomegaly, and autoimmune manifestations. About one-third of patients are asymptomatic at diagnosis and the disease is discovered on a routine blood test showing lymphocytosis and cytopenias, most commonly neutropenia which is present in about 80% of the patients [5, 31]. The pattern and sites of infections are characteristics of neutropenia and include recurrent oral ulcerations [32] and bacterial infections of the skin, oropharynx, pneumonia, and sepsis. Other clinical features are fatigue, B symptoms, arthralgia, and abdominal pain secondary to hepatosplenomegaly. Splenomegaly is present in 20–50% of the patients and contributes to the development of cytopenias by sequestration of mature blood cells, while lymphadenopathy is a rare finding [11]. Anemia is observed in 48% of the cases and about 20% of the patients are transfusion dependent. Laboratory investigations at diagnosis commonly show increased peripheral blood lymphocyte counts between 4 and 10×10^9/l, where the LGL numbers normally range between 1 and 6×10^9/l. Pure red cell aplasia may develop in 8–19% of the patients and mostly moderate thrombocytopenia is found in about 25% of the cases [33]. Clinical chemistry may show elevation of transaminases and alkaline phosphatase reflecting infiltration of the liver by the leukemic cells. Serum protein analyses often demonstrate multiple abnormalities typically observed in autoimmune diseases including polyclonal hypergammaglobulinemia, presence of rheumatoid factor, antinuclear antibodies, and elevated ß2 microglobulin [5, 11]. Less commonly immunofixation electrophoresis reveals the presence of a monoclonal immunoglobulin, indicating the presence of a coexisting B-cell neoplasia, e.g., indolent B-cell lymphoma or monoclonal gammopathy of unknown significance (MGUS) [34, 35].

LGL leukemia is associated with a wide range of comorbid conditions, in particular autoimmune disorders including rheumatoid arthritis occurring in 10–18% of the patients, systemic lupus erythematodes, Sjögren syndrome, and autoimmune thyroid disease [5, 36]. Associations with pulmonary hypertension [37, 38], bone marrow failure syndromes, i.e., aplastic anemia, MDS, and paroxysmal nocturnal hemoglobinuria have also been reported. In two recent studies investigating the B-cell compartment in patients with T-LGL-L 5–27% of the patients showed evidence of a coexisting B-cell LPD, in particular MGUS and CLL [35, 39].

12.5 Etiology and Molecular Pathogenesis of LGL Leukemia

The etiology of LGL leukemias is unknown. As T-LGL cells exhibit phenotypic and molecular features of effector memory cytotoxic T-cells [2, 15], it has been hypothesized that chronic antigen stimulation may play an important role in disease pathogenesis. LGL-L has been linked to viral infections with human T-cell lymphotropic virus and hepatitis C [40]; however, until now a causal role of these infectious agents has not

been convincingly demonstrated. Due to the strong association of T-LGL-L with autoimmune disorders [5, 36, 41] it appears more likely that an as yet unknown autoantigen is the initial activating event resulting in oligoclonal LGL expansion [5]. Continuous upregulation of pro-inflammatory signaling pathways may then favor the emergence of a dominant LGL clone. This concept is supported by longitudinal analyses revealing a shift from oligoclonal to clonal dominance in individual patients [42]. Unexpectedly, the authors of this study also observed a clonal drift, where more than a third of their patients developed a different Vβ T-cell clone over time [42]. It has been suggested that different Vβ clones in an individual patient may recognize different epitopes of the same autoantigen, but so far this interesting hypothesis awaits experimental confirmation [5]. By contrast, recent data obtained from a cohort of 26 T-LGL-L patients demonstrated heterogeneity in the CDR3 regions of the leukemic cell clones arguing against the idea that T-LGL-L is driven by a common (auto) antigen [43].

While the concept of (auto)antigen stimulation remains controversial, it is generally agreed that LGL leukemia is characterized by deregulation of several intracellular signaling pathways including PI3K/Akt, NF-kB, and Fas/Fas ligand that contribute to resistance to apoptosis in the neoplastic cells [5].

Pro-inflammatory cytokines known to be upregulated in LGL-L include interleukin-15 (IL-15) [44], platelet derived growth factor (PDGF) [45], interleukin-6 (IL-6) [46], and chemokines such as CCL3 and CCL5 [47]. These factors are either produced by the neoplastic cells themselves resulting in autocrine stimulation, which has been demonstrated for PDGF [45] or by accessory cells of the tumor microenvironment as has been shown for IL-6 [46]. These proteins exert their growth promoting effects on LGL cells by activating the PI3K-AKT axis and the NF-κB signaling pathway [5, 48, 49]. Interestingly, our group has recently detected inactivating mutations in the NF-κB inhibitor TNFAIP3 (A20) in 3 out of 39 patients (8%) with T-LGL leukemia underscoring the important pathogenic role of deregulated NF-kB signaling in this disease [50].

Under physiologic conditions, when an infection resides, previously activated cytotoxic T-cells including LGL are eliminated through the Fas/FasL pathway in a process which has been termed activation-induced cell death (AICD). Different from normal cytotoxic T-cells neoplastic LGLs are resistant to Fas-mediated apoptosis [51] which has been attributed to increased levels of the proteins c-FLIP and FADD in the leukemic cells. These two factors are important inhibitors of the death-inducing signaling complex (DISC) which is produced in response to the binding of FasL to Fas and believed to play a crucial role in the AICD defect observed in LGL leukemia [52]. The molecular mechanisms underlying overexpression of c-FLIP and FADD remain to be explored. Unlike normal cytotoxic T-cells which express FasL only after activation leukemic LGLs constitutively express FasL on their cell surface, where it can be cleaved by matrix metalloproteinases, thereby producing soluble FasL (sFasL). Serum sFasL levels have been shown to be increased in patients with T-LGL leukemia as compared to healthy blood donors [53]. It has been suggested that both FasL and sFasL bind to Fas on the cell surface of mature granulocytes and their progenitor cells resulting in granulocyte apoptosis [30, 54]. The significance of Fas in mediating cytopenias is further exemplified by the correlation between clinical response to immunosuppressive therapy and declining sFasL serum concentrations [55].

Constitutive activation of JAK/STAT signaling appears to be the most important pathogenic feature of LGL leukemia [5, 25, 56], which was first shown by protein analyses demonstrating overexpression of phosphorylated STAT3 in T-LGL-L as compared to peripheral blood mononuclear cells (PBMC) of healthy donors [22]. These results were confirmed and further extended by a subsequent gene expression profiling revealing upregulation of STAT3 target genes in neoplastic T-LGL cells [25]. As previously mentioned, activation of the JAK/STAT axis is caused by somatic gain-of-function mutations in exon 20 or 21 of the STAT3 gene which can be

observed in 28–75% of the patients [5, 23–25, 56]. Teramo et al. [46] showed that aberrant activation of the JAK/STAT pathway in LGL leukemia may also be caused by IL-6 which is produced by non-neoplastic accessory PBMC. This latter finding explains nicely, why STAT3 activation is also commonly observed in patients with STAT3 wildtype. Thus, aberrant activation of the JAK/STAT axis in LGL cells is caused by both intrinsic and extrinsic factors and appears to be present in the overwhelming majority of patients. This may be also important from the clinical perspective, as STAT3 inhibitors have been shown to reverse the antiapoptotic phenotype of LGL cells in vitro [22].

12.6 Therapy

Approximately one-third of the patients who have moderate cytopenias may never require treatment but clinical follow-up consisting of history, physical examination, and laboratory studies (complete blood count and clinical chemistry parameters including C-reactive protein) is generally recommended [5, 6]. Immunization against pneumococcus and influenza may be effective in preventing in particular respiratory infections which are associated with a high morbidity and mortality in immunosuppressed hematological patients [57]. Neutropenic patients who develop fever should be treated aggressively with anti-infective agents according to published guidelines [58]. Indications for antileukemic treatment include grade 4 neutropenia (absolute neutrophil count [ANC] $<0.5 \times 10^9$/l) with or without life-threatening infections, moderate neutropenia (ANC $>0.5 \times 10^9$/l) associated with recurrent infections, symptomatic anemia or thrombocytopenia, B symptoms, and symptomatic organomegaly. Treatment should also be initiated in rare cases, where infiltration of neoplastic LGL cells into the endothelium or liver parenchyma results in organ dysfunction as evidenced by an increase of liver enzymes or pulmonary hypertension [5, 6].

Immunosuppressive therapy is considered the standard treatment for both T-LGL-L and CLPD-NK. Treatment decisions are currently based on data collected from small case series and only few controlled trials. The most clinical experience has been reported with single agent use of low-dose methotrexate (MTX), cyclophosphamide, and cyclosporine A (CyA) [5, 6]. These drugs have in common that they appear to improve cytopenias in the majority of patients; however, their capacity to eradicate the leukemic cell clone is limited [5, 9, 59]. Therefore, LGL leukemia remains an incurable disease.

Based on retrospective studies either MTX (10 mg/m^2/week), cyclophosphamide (50–100 mg/day), or CyA (3 mg/kg/day) should be used in first-line therapy. Treatment should be continued for a minimum of 4 months before evaluation of response [5, 6, 60, 61]. Traditionally, since the first publication on the efficacy of MTX, this drug has been preferentially used in patients presenting with neutropenia, whereas cyclophosphamide was predominantly utilized in anemic patients. Overall response rates (ORR) range between 21 and 85% and appear to be similar for the three agents [5, 62]. Interestingly, in a French series complete responses (CR) were more commonly observed in patients treated with cyclophosphamide as compared to MTX (33 vs 21%) which also translated into a lower relapse rate (13 vs 67%) [9]. By contrast CyA rarely induces complete responses (<5%) but corrects cytopenias without eliminating the LGL clone [9]. It has been suggested that HLA-DR4 (present in 32% of LGL leukemia) and the STAT3 Y640F mutation may have potential in predicting treatment response to either CyA or cyclophosphamide, respectively [63]. However, the value of these potential biomarkers needs to be validated in larger patient cohorts.

Recently, the results of the first large prospective trial of immunosuppressive agents in LGL leukemia were reported [64]. Fifty-five patients were initially treated with MTX and non-responders were switched to oral cyclophosphamide. The overall response rate to MTX was 38% which is lower than in most retrospective series and 64% in patients receiving cyclophosphamide in the second part of the study [64]. These data are important from the perspective of routine

clinical practice, as they suggest that patients failing a comparably well tolerated treatment with MTX can be safely salvaged with the potentially more toxic cyclophosphamide regimen. However, these data also indicate that cyclophosphamide is more efficient than MTX, which may be particularly true in patients harboring the STAT3 Y640F mutation in their leukemic cell clone. This important question is currently being addressed in an ongoing randomized French trial comparing first-line MTX with cyclophosphamide (NCT01976182) [5].

Based on these data the following recommendations for the treatment of LGL leukemia have been proposed by the two leading groups in the field (Fig. 12.3) [5]. Patients with inadequate response to primary treatment should be switched between MTX and cyclophosphamide. CyA is reserved for patients failing both drugs. MTX and CyA are dosed continuously until either intolerance or resistance to the drug occurs. It is important to closely monitor the side effects of these medications. Common adverse events reported for the long-term use of MTX include hepatitis and pneumonitis [65], while patients undergoing treatment with CyA should be monitored for renal function, arterial hypertension, and gingival hyperplasia. Because of its mutagenic side effects it is recommended to stop cyclophosphamide therapy after 8–12 months [5].

Because of the rarity of the disease and the scarcity of prospective data, it is even more difficult to make treatment recommendations in the relapse/refractory setting. To our knowledge there is only data from one prospective phase II trial evaluating the efficacy and safety of alemtuzumab in pretreated patients that have been recently published [66]. Twenty-five patients with T-LGL-L enrolled between 2006 and 2015 received the anti-CD52 antibody at 10 mg/day intravenously for 10 consecutive days of a single treatment course. In the intention to treat analysis the hematological response rate at 3 months was 56%. In the responding patients alemtuzumab effectively reduced the number of leukemic cells but failed to completely eradicate the neoplastic cell clone. Toxicities were manageable and generally restricted to first dose infusion reactions and subclinical EBV and CMV reactivations. Thus, alemtuzumab is an option in this difficult

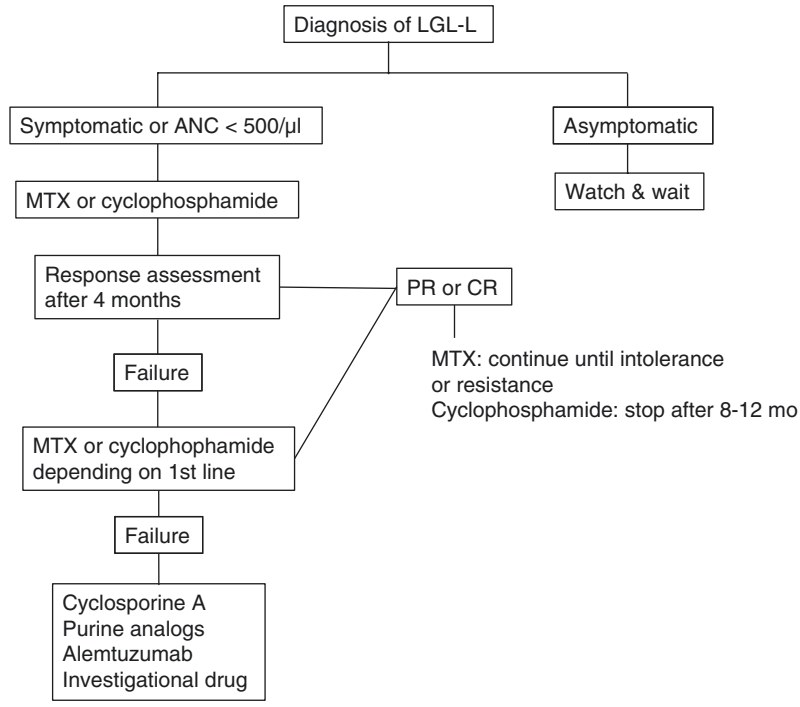


Fig. 12.3 Treatment algorithm. Adopted from [5, 6]

to treat patient population [66, 67]. However, in view of the limited access to alemtuzumab and the rigorous requirements for supportive care and viral monitoring, this treatment should be restricted to centers with clinical experience in the use of this antibody.

Published data on the activity of purine analogs including fludarabine, cladribine, deoxycoformycine, and bendamustine are limited to fewer than 50 patients. However, these small series demonstrated remarkable ORR of >70% and in some cases long-lasting complete remissions [5, 68, 69]. In multirefractory patients autologous or allogeneic hematopoietic stem cell transplantation (HSCT) has been used. The European Society for Bone and Marrow Transplantation (EBMT) recently reported their results from a registry-based retrospective study of 15 patients (allo-HSCT 10 and auto-HSCT 5) [70]. Patients with chemosensitive disease autografted in first complete remission appeared to have benefited with long-lasting remissions. Durable remissions were also observed in patients receiving an allograft even if they had been transplanted with non-remission advanced disease, suggesting a graft versus LGL effect. Five patients in the allo-HSCT group were alive and in remission after a median follow-up of 30 months accounting for a 2-year progression free survival (PFS) and overall survival (OS) of 50% each [70]. Thus, although these results need to be interpreted with caution due to the small number of cases analyzed, HSCT appears to be feasible and effective in a small subgroup of relapsed LGL leukemia patients presenting with an aggressive and/or refractory form of their disease [5, 70].

Based on single-center experiences, supportive treatment with granulocyte colony-stimulating factor (G-CSF) can be a potentially life-saving option in T-LGL patients with neutropenic fever complicated by sepsis and/or severe pneumonia where a rapid increase of neutrophils is important. However, data regarding the utility of G-CSF in this situation are sparse and such treatment may induce articular pain and an exacerbation of splenomegaly [71, 72]. There is evidence that intermittent G-CSF administration is of value in patients with Felty's syndrome with severe neutropenia and some of these series also included patients with T-LGL [73]. However, it should be noted that this approach is merely symptomatic in nature and does not target the underlying malignant disease.

Splenectomy may be used in patients with symptomatic splenomegaly and inadequate response to immunosuppressive treatment. Retrospective case series reported an acceptable safety profile and improvement of pain and cytopenias in the majority of patients [5, 74]. However, response durations appeared to be limited and again small patient numbers preclude any systematic evaluation of this approach.

Considering our recently improved understanding of the molecular pathogenesis of LGL-L, the development of treatment modalities targeting specific deregulated signaling pathways appears promising. Unfortunately, clinical studies employing inhibitors of IL-15 [75] and farnesyltransferase [76] did not show clinical responses. Until now, the most encouraging results could be observed in patients undergoing treatment with the JAK3 inhibitor Tofacitinib, which has recently been approved for the immunosuppressive treatment of refractory rheumatoid arthritis (RA). Bilori et al. [77] observed hematological improvements in 6 of 9 patients suffering from both RA and T-LGL, suggesting the possibility that JAK/STAT inhibitors may have potential as a new salvage therapy for refractory T-LGL.

12.7 Recommendations for Post-Treatment Follow-Up

Data on the post-treatment follow-up in patients with T-LGL are limited. Thus, recommendations for clinical practice may be informed by studies in more common lymphoproliferative diseases such as CLL [78] and patients with autoimmune disorders exposed to long-term immunosuppression [79]. Similar to CLL LGL-L can be considered an incurable disease in most patients and thus life-long observation and follow-up are justified. Follow-up of asymptomatic patients should include a blood cell count, physical exam-

ination, and ultrasound scans of the abdomen every 3–12 months depending on the dynamics of the disease. Careful palpation of peripheral lymph nodes and screening for monoclonal serum immunoglobulins should be performed to detect potentially developing B-cell LPDs which complicate the natural course of LGL-L in up to 27% of the patients [34, 35, 39]. Recent studies have documented the risk of hepatitis B (HBV) reactivation during immunosuppressive therapy [80], although, at least to our knowledge, there have been no reports of its occurrence in LGL-L. In view of the high morbidity and mortality of HBV reactivations in patients with LPD receiving chemo- or immunotherapy, screening for HBV and preemptive treatment with lamivudine or entecavir is also routinely performed in patients with LGL-L at our center. In general, the time intervals and extent of clinical follow-up investigations should be tailored to the individual patient's history, infectious complications, intensity of immunosuppression, and treatment modality. For example the minority of patients with relapsed and refractory disease treated with purine analogs or alemtuzumab require more intensive surveillance and anti-infective prophylactic measures than individuals undergoing first-line therapy with MTX, CSA, or cyclophosphamide. On the other hand long-term cyclophosphamide therapy is associated with a prolonged risk of specific secondary malignancies including non-melanoma skin cancer, bladder cancer, and most importantly myeloid leukemias [79]. The risk of myeloid malignancies has been found to be particularly high in patients receiving a cumulative cyclophosphamide dose of more than 36 g and the leukemia incidence increases significantly 5–9 years after the initiation of treatment [79].

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