Chronic Lymphocytic Leukemia

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Patients with chronic lymphocytic leukemia can be divided into three categories: those who are minimally affected by the problem, often never requiring therapy; those that initially follow an indolent course but subsequently progress and require therapy; and those that from the point of diagnosis exhibit an aggressive disease necessitating treatment. Likewise, such patients pass through three phases: development of the disease, diagnosis, and need for therapy. Finally, the leukemic clones of all patients appear to require continuous input from the exterior, most often through membrane receptors, to allow them to survive and grow. This review is presented according to the temporal course that the disease follows, focusing on those external influences from the tissue microenvironment (TME) that support the time lines as well as those internal influences that are inherited or develop as genetic and epigenetic changes occurring over the time line. Regarding the former, special emphasis is placed on the input provided via the B-cell receptor for antigen and the C-X-C-motif chemokine receptor-4 and the therapeutic agents that block these inputs. Regarding the latter, prominence is laid upon inherited susceptibility genes and the genetic and epigenetic abnormalities that lead to the developmental and progression of the disease.

Chronic lymphocytic leukemia (CLL) is a disease of aging adults. Because it often begins as a relatively indolent condition with many patients having long survival, CLL has a high prevalence rate, making it the most common adult leukemia in western countries. The disease results from the overgrowth of a single CD5⁺ B lymphocyte co-expressing low levels of surface membrane immunoglobulin (smIg) of a single IG light (L) chain type and of CD79b, CD20, and CD23. The clinical consequences of this clonal overgrowth are highly variable: Some patients die within 2–3 yr of diagnosis, whereas others survive decades beyond. This variability

is due to factors intrinsic to the leukemic B cell (e.g., genetic and epigenetic changes in coding and noncoding genes) and factors extrinsic to the leukemic cell (e.g., inputs delivered by various signaling pathways in the tissue microenvironment [TME]).

This perspective is organized according to the order CLL evolves in patients. It starts with epidemiologic, genetic, and environmental factors that influence development of the disease, follows with features leading to the clinical presentation, diagnosis, and initial treatment, if necessary, and concludes with elements causing disease progression or therapy refractoriness,

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describing current and emerging therapeutic options. Emphasis is placed on recent findings relating to these three periods.

DISEASE DEVELOPMENT

Epidemiology

According to the National Cancer Institute's Surveillance, Epidemiology, and End Results Program (SEER), the estimated number of new cases of CLL in the United States in 2018 was 20,940, representing ~1.2% of new cancer diagnoses and the number of deaths from CLL was 4510, ~0.7% of all cancer deaths (SEER Cancer Stat Facts: Chronic Lymphocytic Leukemia, National Cancer Institute, Bethesda, MD; <https://seer.cancer.gov/statfacts/html/clyl.html>). Median age at diagnosis was 70, with the highest numbers of cases identified in the 65–74 yr age group.

However, these statistics are not relevant worldwide as the incidence of CLL varies based on race/ethnicity: White > Black > Hispanic > Asian/Pacific Islander. Incidence also varies based on gender, being approximately two-fold more frequent in males.

Environmental Considerations

Exposure to pesticides, specifically deltamethrin (Leon et al. 2019), and herbicides (Alavanja et al. 2014; Coggon et al. 2015) has been associated with the development of CLL; the most convincing is Agent Orange used during the Vietnam War (Baumann Kreuziger et al. 2014; Mescher et al. 2018). Radon exposure has shown a similar relationship (Schwartz and Klug 2016).

The link with ionizing radiation is controversial. Because CLL incidence did not increase among Japanese exposed to atomic bomb blasts in World War II (Preston et al. 1994), ionizing radiation was not considered a risk factor. However, epidemiologic studies of people in the vicinity of the Grenoble nuclear power plant breakdown suggest an increased incidence among exposed individuals (Gluzman et al. 2006; Chumak et al. 2008; Kesminiene et al. 2008; Romanenko et al. 2008; Zablotska et al. 2013), so this conclusion needs reconsideration.

Finally, respiratory tract infections, cellulitis, and herpes zoster can presage CLL (Landgren et al. 2007a,b; Lesley et al. 2009), implying an underlying immune defect predisposing to disease development. Consistent with this, hypogammaglobulinemia is common in CLL and can precede diagnosis (Lenders et al. 1984; Tsai et al. 2009).

Genetic Considerations

Inheritance of Susceptibility Genes

CLL has the highest incidence of familial association among leukemias, with first-degree relatives having more than eight-fold higher likelihood of developing the disease (Goldin et al. 2004). Conversely, the low CLL incidence among Asians/Pacific Islanders, particularly Japanese, does not increase when living in the United States (Gale et al. 2000). Both observations strongly imply involvement of inheritable susceptibility alleles that promote or prevent disease. Genome-wide association studies (GWASs) identified several polymorphic genetic loci (Di Bernardo et al. 2008; Crowther-Swanepoel et al. 2010a; Slager et al. 2011, 2012; Berndt et al. 2013, 2016; Speedy et al. 2014). These have been refined to the nine most likely (Law et al. 2017), which are transcriptionally active in CLL cells and contain genes involved in the control of human B-cell development and signaling or of immune function (Law et al. 2017). Consistent with this, GWASs of CLL and myeloma patients uncovered shared risk loci influenced by polymorphisms in B-cell regulatory elements affecting genes involved in B-cell development (Went et al. 2019).

Normal B-lymphocyte development proceeds by an ordered process of cellular maturation occurring in the bone marrow (BM), beginning with a hematopoietic stem cell (HSC) and culminating with a mature B cell (Fig. 1A). Mature B lymphocytes evolve to memory and/or plasma cells based on the types of antigens encountered in the periphery and the maturation pathways they are guided to follow by cells of the hematopoietic and nonhematopoietic lineages. Thus, susceptibility genes can exert in-

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fluences at many points along the B-cell differentiation path.

Noninherited Recurrent Genetic Mutations in CLL Cells

The leukemic cells from the majority of patients exhibit chromosomal abnormalities at some point in the course of disease. Moreover, normal cells at early stages of hematopoiesis taken from CLL patients have mutations present in their leukemic clone (Damm et al. 2014; Marsilio et al. 2018). Transplantation of HSCs from CLL patients into immune-compromised mice leads to mature CD5⁺ B cells resembling those in CLL patients (Kikushige et al. 2011). These early mutations, however, are not sufficient to yield the full-fledged disease (Kikushige et al. 2011) but result in a pre-leukemic state resembling monoclonal B lymphocytosis (Rawstron et al. 2002b).

The most common somatic abnormalities found in mature CLL cells are del13q, tri12, del11q, and del17p, in this order of frequency

(Döhner et al. 2000; Haferlach et al. 2007; Zenz et al. 2010a; Stilgenbauer 2014). Deletions at 13q and tri12 are found at diagnosis, suggesting these are initiating events; del11q and del17p are most often found later in disease, implying they promote clonal evolution and disease progression. The incriminating genetic elements in del13q are two microRNAs, *miR15a/16b* (Calin et al. 2002). Loss of *miR15a/16b* leads to overproduction of the anti-apoptotic protein Bcl-2 (Cimmino et al. 2005) and heightened cell-cycle progression (Klein et al. 2010), both oncogene-like effects. Moreover, mice with a defective *miR15/16* spontaneously develop a CLL-like disease (Raveche et al. 2007; Hayakawa et al. 2016), and deletion of the chromosomal region corresponding to 13q in mice leads to murine CLL (Klein et al. 2010; Kasar et al. 2016).

The advent of high-throughput, next-generation deep DNA sequencing enabled the demonstration that CLL cells also exhibit specific gene aberrations. Beginning in 2011 (Fabbri et al. 2011; Puente et al. 2011; Quesada et al.

Figure 1. (Continued.) Microenvironment-supported B-lymphocyte development in patients with chronic lymphocytic leukemia (CLL). (A) Differentiation scheme assumed for CLL and Richter's transformation (RT) cells. Normal B lymphocytes progress through an ordered differentiation program that begins with hematopoietic stem cells (HSCs), proceeds through multipotent progenitor cells (MPPs), and then common lymphoid precursors (CLPs). After this point, commitment is made to the B-lymphocyte lineage. Pro-, pre-, and immature B cells differ based on the progressive rearrangements of *IGHV*, *IGHD*, and *IGHJ* genes. *IGHD-IGHJ* rearrangement is completed in pro-B cells, followed by *IGHV-IGHD-IGHJ* rearrangement in pre-B cells, and both *IGHV-IGHD-IGHJ* + *IGLV-IGLJ* rearrangements in immature B cells. Thus, antibodies that can create a complete B-cell receptor (BCR) exist from the pre-B cell-immature B cell interphase and at later stages of B-cell development (transitional, mature, etc.). Mutations in CLL HSCs and MPPs have been documented (Damm et al. 2014; Marsilio et al. 2018), and xenografting CLL HSCs/MPPs into severely immune-compromised mice (Kikushige et al. 2011) leads to a condition resembling monoclonal B-lymphocytosis (Rawstron et al. 2002a). If leukemia stem cells exist in CLL, HSCs, MPPs, CLPs, and pro-B cells carrying genetic abnormalities would be considered "pre-leukemic stem cells" and not "leukemic stem cells," because they could not give rise to a CLL cell with the same *IGHV-IGHD-IGHJ* + *IGLV-IGLJ* rearrangements. The latter is relevant because, upon disease relapse, the emerging cells express a complete B-cell receptor (*IGHV-IGHD-IGHJ* + *IGLV-IGLJ* rearrangements) that indicates it is a member of the original CLL clone. Hence, we suggest the true leukemic stem cell in CLL resides at a differentiation stage at or beyond the pre-B cell-immature B cell interphase. CLL cells proliferate in secondary lymphoid organs and can transform to Richter's cells, often as CLL-derived diffuse large B-cell lymphoma (DLBCL) that have larger cells with prominent nuclei. (B) Microenvironmental signals associated with clonal expansion. (Left) Bone marrow microenvironment that supports normal and abnormal hematopoietic development. Bone marrow niche supports the differentiation and maturation of B cells. Immature HSCs and B cells are maintained and regulated by niche factors (i.e., CXCL12 and SCF produced by MSC), endothelial cell-derived signals (i.e., SCF, E-selectin, JAGGED1), and the progeny of HSCs including macrophages and megakaryocyte and regulatory T cells. (Right) The secondary lymphoid microenvironment sustains CLL and RT cells. CLL and RT cells receive growth promotion (e.g., IL-4, IL-21, CD40L, CCL19) and suppression (e.g., IL-10, IL-35, and PD1-PD1 interaction) signals, as well as cell homing and retention cues (CXCL12, CXCL13, and CD31). Th2, T helper 2 cells; FDC, follicular dendritic cell; NK, natural-killer cell; MSC, mesenchymal stromal cell; Treg, T regulatory cell.

2011; Wang et al. 2011) and now involving approximately 1000 CLL exomes (Quesada et al. 2012; Landau et al. 2013, 2015; Puente et al. 2015; Ljungström et al. 2016), a series of gene mutations was identified in CLL, although not as plentiful as in solid tumors (Table 1; Pleasance et al. 2010). Most of these lead to amino acid changes, suggesting selection for pathogenicity. Pathways controlling DNA damage repair/cell cycle control, Notch signaling, IL-1R family signaling, Wnt signaling, and RNA processing and export, MYC activity, and MAPK signaling are affected (Wang et al. 2011; Landau et al. 2015).

More disease-driving aberrations are found in *IGHV*-unmutated CLL (U-CLL) than in *IGHV*-mutated CLL (M-CLL). Additionally, certain mutations occur more frequently in U-CLL (*NOTCH1*, *XPO1*, and *POT1*) and others in M-CLL (*del*(13q), *MYD88*, and *CHD2*), or in

CLL subsets with specific genomic aberrations (e.g., *MYD88* with *del*(13q); *SF3B1* with *del*(11q); *NOTCH1*, *BIRC3*, *FBXW7*, and *BCOR* with *tri*12 [Table 1]). Moreover, sets of mutations associate with the use of certain stereotyped *IGHV-IGHD-IGHJ* gene rearrangements (Rossi et al. 2009b; Sutton et al. 2016). Selected mutations occur in specific regions of individual genes (e.g., PEST domain of *NOTCH1* and HEAT domain of *SF3B1*). In some instances, a relationship exists between a specific abnormality and aggressive clinical course and/or shortened survival (Rossi et al. 2012a); this might be caused by development of Richter's transformation (RT) (Richter 1928) (Table 2; Fabbri et al. 2011; Rossi et al. 2012b).

Collectively, these associations and the fact that no single mutation is found in every CLL clone strongly suggest the development of CLL differs for distinct subsets of patients. In this

Table 1. Recurrent mutations in chronic lymphocytic leukemia (CLL) B cells that associate with chromosomal aberrations, *IGHV* genes, and mutation status and stereotyped subsets

Mutation	Chromosomal aberrations ^a	<i>IGHV</i> gene ^b	Stereotyped subset ^c
<i>SF3B1</i>	<i>del</i> 11q <i>del</i> 13q	<i>IGHV1-69</i> (unmutated) <i>IGHV3-21</i> (unmutated) <i>IGHV3-21</i> (mutated)	#3, #7 #2 #2
<i>ATM</i>	<i>del</i> 11q <i>del</i> 13q	<i>IGHV3-21</i> (unmutated) <i>IGHV3-21</i> (mutated)	#2 #2
<i>NOTCH1</i>	Trisomy 12	<i>IGHV1-69</i> (unmutated) <i>IGHV4-39</i> (unmutated) Clan I genes (unmutated)	#6 #8 #1, #99, #59,
<i>BCOR</i>	Trisomy 12		
<i>BIRC3</i>	Trisomy 12		
<i>FBXW7</i>	Trisomy 12		
<i>XPO1</i>	<i>del</i> 11q <i>del</i> 13q	U-CLL > M-CLL	
<i>POT1</i>	<i>del</i> 11q <i>del</i> 13q	U-CLL > M-CLL	
<i>CHD2</i>	<i>del</i> 13q	M-CLL > U-CLL	
<i>TP53</i>	<i>del</i> 13q <i>del</i> 17p	Clan I genes (unmutated)	#1, #99
<i>MYD88</i>	<i>del</i> 13q	M-CLL > U-CLL	
<i>SAMHD1</i>	<i>del</i> 13q		

^aMain recurrent genetic aberrations associated with chromosomal abnormalities of known prognostic significance in CLL.

^b*IGHV* (immunoglobulin heavy variable) gene and mutation status associated with common genetic aberrations. *IGHV* gene mutations are determined based on degree of identity with the germline ($\geq 98\%$ identity = "unmutated," U-CLL; $< 98\%$ germline identity = "mutated," M-CLL). Mutations of *XPO1*, *POT1*, *CHD2*, and *MYD88* are not associated with a specific *IGHV* gene mutation but occur more frequently in M-CLL or U-CLL.

^cStereotyped subset-biased acquisition of recurrent gene mutations. *MYD88* and *BIRC3* mutations are rare in all subsets.

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Table 2. Biological pathways associated with common mutations

Type of pathway	Common mutations
RNA and ribosomal-processing	<i>SF3B1, XPO1, RPS15, DOX3X, ZNF292, MED12, NXF1</i>
DNA-damage and cell-cycle-control	<i>ATM, TP53, POT1</i>
Chromatin-modification	<i>CHD2, ZMYM3, BAZ2A, ASXL1, SETD2</i>
Notch-signaling	<i>NOTCH1, FBXW7</i>
Inflammatory	<i>BIRC3, MYD88, TRAF3, SAMHD1</i>
MAPK-ERK	<i>BRAF, KRAS, MAP2K1, NRAS</i>
WNT-signaling and MYC-related	<i>MGA, PTPN11</i>
BCR-signaling	<i>EGR2, PAX5, BCOR, IRF4, IKZF3</i>

MAPK, Mitogen-activated protein kinases; ERK, extracellular signal-regulated kinases; WNT, wingless/integrated; BCR, B-cell receptor.

regard, mutations in U-CLL patients are more often found in coding regions of driver genes, and mutations in M-CLL tend to target promoter and enhancer regions. Moreover, the former mutations carry signatures suggesting a mutation mechanism associated with aging, whereas the latter suggest a mechanism associated with activation-induced cytidine deaminase (AID) activity (Burns et al. 2018). These mutational mechanisms have been suggested as active in CLL, albeit not in an *IGHV*-defined subset setting (Alexandrov et al. 2013; Kasar et al. 2015).

Influences of B-Lymphocyte Surface Receptors on CLL

In addition to genetic and environmental factors, normal B lymphocytes are influenced by cell-autonomous and cell-extrinsic signals that promote clonal survival and expansion.

Signals Initiated by the B-Cell Receptor for Antigen

Normal B lymphocytes respond to insults (often microbial) from the exterior and from within. In each instance, engagement of (auto)antigens by the smlg of the B-cell receptor (BCR) transmits an activating signal leading to survival and growth or anergy or death (Fig. 2). Based on structural studies of variable domains of Igs made by the leukemic B lymphocytes, the (auto)antigens they can bind, and the consequences of initiating such signals in vitro, it appears CLL B cells respond similarly to such inputs.

Consistent with derivation from a human B-cell subpopulation analogous to the murine CD5⁺/Ly1⁺/B1⁺ B-cell subset (Hayakawa et al. 1983), initial studies of the genes coding for the variable domains of CLL Igs suggested these were restricted in use (Fong et al. 1985; Kipps et al. 1988) and were minimally, if at all, different from the germline sequence (Meeker et al. 1988; Pratt et al. 1989; Küppers et al. 1991; Friedman et al. 1992; Wagner and Luzzatto 1993; Pan et al. 1996). Subsequent studies confirmed restricted gene use (Johnson et al. 1997; Fais et al. 1998; Widhopf and Kipps 2001; Kröber et al. 2002; Ghiotto et al. 2006; Hadzidimitriou et al. 2009), although a review of available CLL sequences (Schroeder and Dighiero 1994) and prospective analyses of IgG⁺ (Hashimoto et al. 1995) and IgM⁺ (Fais et al. 1998) clones showed that >50% of randomly chosen IgM⁺ CLL cases as well as ~75% of IgG⁺ and IgA⁺ cases exhibited significant numbers of *IGHV* mutations (>2% differences from the most similar germline gene). Thus, CLL clones are molecularly heterogeneous and can be subgrouped based on *IGHV* gene mutations. Notably, these two CLL types, M-CLL and U-CLL, have dramatically different clinical courses and outcomes (Damle et al. 1999; Hamblin et al. 1999), and segregating CLL patients this way is a valuable prognostic indicator for patient outcome.

Furthermore, certain CLL Igs have remarkably similar amino acid *IGHV-IGHD-IGHJ* rearrangements with characteristic HCDR3 amino acid motifs (Tobin et al. 2003, 2004; Ghiotto et al. 2004; Messmer et al. 2004; Stamatopoulos

Figure 2. B-cell receptor (BCR) signaling pathway and the effects of kinase inhibition. Upon antigen interaction with the membrane IG component of the BCR, Lyn is activated and in turn phosphorylates CD79 on tyrosine residues—in particular, those in ITAM motifs. This permits binding of SYK and various adaptor molecules that initiate signaling that proceeds through key kinases including BTK, PI3Kδ, and AKT. (For a comprehensive review, see Slupsky [2014].) The sites of action for kinase inhibitors are indicated: BTK (ibrutinib), PI3Kδ (idelalisib), PI3Kδ+γ (duvelisib), and SYK (fostamatinib).

et al. 2007, 2017; Vardi et al. 2014; Agathangelidis et al. 2019); these are referred to as “stereotyped BCRs” (Messmer et al. 2004). The likelihood such rearrangements occur by chance is infinitesimally small (10^{-12}), indicating selection of normal B lymphocytes with defined and in some cases quasi-unique BCR structures rarely found in large amounts in the normal B-cell repertoire. Stereotyped BCRs are found in ~33% of CLL cases (Stamatopoulos et al. 2007; Murray et al. 2008; Agathangelidis et al. 2012, 2019).

When considering U-CLL, ~50% of cases fall into a stereotyped subset.

Foreign or autologous antigens engage smIg leading to a series of stimulatory events activating various B-cell fates: survival, expansion, anergy, or death. The state or type of B cell as well as concomitant signals delivered via membrane molecules on other cells or their secreted factors determine which fate occurs. For CLL cells, the antigens or classes of antigens involved are foreign (e.g., viral [Steininger et al. 2012], fungal

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[Hoogeboom et al. 2013], and bacterial [Hatzi et al. 2016]) or autologous (e.g., self-antigens characteristic of autoimmune disorders [Bröker et al. 1988; Sthoeger et al. 1989; Borche et al. 1990; Hervé et al. 2005]) and self-antigens developing during apoptosis or normal catabolism (Catera et al. 2006; Chu et al. 2008, 2010; Lanemo Myhrinder et al. 2008). B-cell interactions with antigens that support survival and expansion most likely occur in solid tissues where most CLL cells divide (Calissano et al. 2011; Herishanu et al. 2011; Herndon et al. 2017) and hematopoietic and nonhematopoietic cells support survival and expansion.

Another important type of autoreactivity characteristic of CLL IGs is their ability to bind themselves (Binder et al. 2011, 2013; Dühren-von Minden et al. 2012), leading to self-association/homodimerization (Minici et al. 2017). This interaction results in BCR signaling without binding antigenic epitopes external to the B cell and extrinsic to the BCR, referred to as “autonomous signaling” (Dühren-von Minden et al. 2012). Although not yet studied thoroughly, the affinity of self-association, and hence the frequency of autonomous signaling, correlates with patient outcome: High affinity correlates with better outcome and low affinity with bad outcome (Minici et al. 2017). It has been suggested that this type of signaling promotes survival or anergy, whereas signaling resulting from smIg binding of non-IG antigens leads to proliferation (Chiorazzi and Efremov 2013). Self-association/autonomous signaling occurs in normal pre-B cells (Köhler et al. 2008; Eschbach et al. 2011) and B-1 cells in mice (Köhler et al. 2008), and findings from a mouse model of CLL support its importance in the human disease (Iacovelli et al. 2015).

Signals Initiated by Other Membrane-Associated Pathways

Several other signaling pathways affect the biology of CLL cells. The C-X-C-motif chemokine receptor-4 (CXCR4) is critical in allowing CLL cells to traffic to survival niches in solid tissues (Burger and Bürkle 2007) and delivers survival signals to normal B lymphocytes and CLL cells

(Burger and Kipps 2002). Similarly, CD38 and CD49d, two membrane molecules whose levels of expression correlate with clinical course in CLL (Damle et al. 1999; Bulian et al. 2008; Gattei et al. 2008; Rossi et al. 2008; Shanafelt et al. 2008a), deliver trophic signals to CLL cells (Deaglio et al. 2003, 2007; Zucchetto et al. 2009, 2012; Vaisitti et al. 2010). The orphan receptor ROR-1, initially identified by gene expression analyses of CLL B cells (Klein et al. 2001; Rosenwald et al. 2001) and subsequently found on CLL cells (Baskar et al. 2008; Daneshmanesh et al. 2008; Fukuda et al. 2008), delivers signals, either constitutively (Hojjat-Farsangi et al. 2013) or after interacting with Wnt family ligands (Yu et al. 2016). Because ROR-1 signaling promotes CLL cell survival, growth, and migration (Cui et al. 2016; Hasan et al. 2017, 2019; Yu et al. 2017) and ROR-1 is expressed only on CLL cells and certain other B-cell lymphoproliferative disorders and not on immature and mature normal B lymphocytes (Broome et al. 2011), targeting the molecule is a potential therapy (Yang et al. 2011). Clinical trials with anti-ROR-1 monoclonal antibodies (mAbs) (Choi et al. 2015) and with chimeric antigen-receptor (CAR)-T cells bearing anti-ROR-1 mAbs (Hudecek et al. 2010) are under way.

Other Microenvironmental Signals Associated with Clonal Expansion

Other hematopoietic and nonhematopoietic cells foster clonal expansion, either by cell–cell contact or by elaborating cytokines and chemokines (Fig. 1B). For example, nurse-like cells, a CLL equivalent of tumor-associated macrophages (Tsukada et al. 2002), secrete the CXCR4 ligand, CXCL12; this attracts leukemic B cells to these trophic elements that secrete BAFF and APRIL (Nishio et al. 2005). Additionally, classical macrophages, particularly those of the M2 type, promote survival, and elimination of these cells prevents CLL-cell growth (Galletti et al. 2016; Hanna et al. 2016). In addition, although T lymphocytes in CLL are defective in interacting with leukemic B cells (Ramsay et al. 2008) and hence do not effectively carry out cytotoxicity, they do promote survival and expansion in vitro (Granziero et al. 2001; Os et al. 2013) and

in xenografts (Bagnara et al. 2011; Patten et al. 2016). CLL cells, especially from poor-outcome U-CLL patients, secrete CCL3 and CCL4 that draw T cells into their vicinity, thereby receiving trophic signals (Burger et al. 2009). IL-4 is especially relevant for survival and reprogramming smIg expression (Fig. 3; Aguilar-Hernandez et al. 2016; Guo et al. 2016). Finally, mesenchymal stromal cells support CLL-cell survival by secreting cytokines and chemokines (Trimarco et al. 2015), as do vascular endothelial cells expressing CD31, the co-receptor for CD38 on hematopoietic cells including CLL cells (Deaglio et al. 1996, 2000).

B lymphocytes bearing a CLL phenotype can be found in the blood of normal individuals, in some instances years before the diagnosis of CLL (Landgren et al. 2009; Georgiadis et al. 2017). This condition, termed monoclonal B-cell lymphocytosis (MBL), is defined by elevated numbers of CD5⁺CD19⁺CD20^{low}CD79b^{low}Ig^{low} cells in the blood and no evidence for CLL or small lymphocytic lymphoma (SLL) (Marti et al. 2005; Shanafelt et al. 2010).

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age of 40 has MBL, increasing to ~15% after the age of 70 (Rawstron et al. 2002a; Ghia et al. 2004). Moreover, first-degree relatives in familial CLL cohorts have a ~15% incidence of MBL after the age of 40, and first-degree relatives in families with sporadic CLL have a similar incidence after age 60 (Rawstron et al. 2002b; Marti et al. 2003; Matos et al. 2009; Goldin et al. 2010). Because annually only 1% of subjects with MBL progress to CLL requiring therapy (Rawstron et al. 2002a,b; Fung et al. 2007; Rossi 2009a; Shanafelt et al. 2009b; Molica et al. 2011), additional events, most likely genetic aberrations, are needed to usher this common condition to leukemia. Elevated serum β_2 -microglobulin levels and expression of an unmutated *IGHV* by the MBL clone predict a shorter transformation time to CLL (Parikh et al. 2018).

The common genetic abnormalities (Rawstron et al. 2008; Shanafelt et al. 2009a; Molica et al. 2011) and susceptibility loci (Crowther-Swanepoel et al. 2010b) characteristic of CLL are found in MBL. Although limited, genetic studies using next-generation sequencing (NGS) reveal similarities between MBL and early-stage CLL (Rasi et al. 2012; Greco et al. 2013; Ojha et al. 2014; Agathangelidis et al. 2018; Popp et al. 2019). MBL and CLL samples have similar mutation burdens; however, when analyzed for specific driver mutations, less are found in MBL (Puente et al. 2015), consistent with the stepwise evolution from one to the other.

Finally, as in CLL, there are implications for a more global immune defect in people with MBL. Such individuals have a higher incidence of severe infections (Lesley et al. 2009; Casabonne et al. 2012; Moreira et al. 2013) and non-hematologic cancers (Solomon et al. 2016) and may have an increased incidence of hypogammaglobulinemia (Glancy and Siles 2016).

Normal Cell Equivalents of Full-Fledged CLL B Cells

CLL cells are identified by CD5 expression and hence could be derived from a normal CD5⁺ human B lymphocyte. It is believed that this human B-cell subset is evolutionarily aligned with the murine B-1 subset (Hayakawa et al. 1983). Al-

though murine CD5⁺ B-1 cells have been extensively studied, it is controversial whether they derive from a one-of-a-kind hematopoietic precursor, hence embodying a distinct B-cell lineage (Montecino-Rodriguez et al. 2006; Ghosn et al. 2011), or represent sets of B lymphocytes that acquire a unique surface membrane phenotype and functional features based on the maturational path followed during development (Houghton et al. 1993). The same conundrum exists for CLL—that is, one precursor cell type that follows different maturation stages, thereby accumulating or not somatic *IGHV* mutations, or two (or more) fundamentally distinct precursors. Candidate precursors include mature CD5⁺ B cells (Seifert et al. 2012) and a B-cell subset bearing the CD20⁺CD27⁺CD43⁺CD70⁻ surface membrane phenotype (Griffin et al. 2011).

Because CLL B cells fall into two categories based on *IGHV* gene mutations, it has been suggested U-CLL cells derive from naive B lymphocytes and M-CLL from memory B cells. However, because both CLL subtypes appear to be chronically activated cells based on membrane phenotype (Damle et al. 2002), telomere lengths (Damle et al. 2004; Strefford et al. 2015), gene expression profiling (Klein et al. 2001; Rosenwald et al. 2001), and epigenetic analyses (Oakes et al. 2016), precursors for both CLL subtypes are likely antigen-experienced. In this regard, because U-CLL clones can spontaneously (Gurrieri et al. 2002; Bagnara et al. 2006) or upon stimulation (Patten et al. 2012, 2016) acquire new *IGHV* mutations, it is possible the lack of mutations in the majority of cells in these clones is due to the developmental path the normal B cell followed prior to transformation (Chiorazzi and Ferrarini 2003).

Collectively, the above suggests CLL develops in genetically susceptible people upon exposure to still ill-defined environmental antigens possibly acting in concert with common autoantigens. This might be a stepwise process beginning early in hematopoietic and B-cell ontogeny and ending in cells downstream in the B-cell developmental pathway at a point after rearrangement of the *IGHV-IGHD-IGHJ* and *IGLV-IGLJ* segments (Fig. 1A). This selection is mediated either by classical interactions of

smIg with (auto)antigens or by smIg self-association and consequent autonomous signaling. Chronic stimulation over time, probably due to ongoing stimulation through the BCR and possibly other pathways (e.g., TLR9 or TLR7), leads to repetitive rounds of DNA replication and development of genetic abnormalities that complement each other and create a leukemic clone. This occurs in normal B cells selected for transformation based on the structure of their BCRs.

DIAGNOSIS AND INITIAL TREATMENT OF CLL

Clinical and Laboratory Parameters

In most western countries in which health care is readily accessible, diagnosis of CLL is made during a routine medical visit by finding an elevated number of lymphocytes on a complete blood count. If lymphocyte expansion persists, flow cytometric analysis of the number of CD5⁺CD19⁺ B cells is carried out, searching for an increased number of cells with low levels of smIg of a single IG L chain type co-expressing CD20, CD79b, and CD23. Because most patients are asymptomatic at this time, clinical history is often not revealing, although weight loss, lethargy, night sweats, and complaints of “swollen glands” may be reported. Physical examination focuses on identifying lymphadenopathy and hepatosplenomegaly and evidence of red blood cell or platelet deficiencies. Guidelines for the diagnosis and treatment of CLL have recently been updated (Hallek et al. 2018).

Prognostic Markers

Upon diagnosis of CLL, a series of prognostic indicators may assist in defining the stage of the disease and in predicting the clinical course that an individual patient will follow. Clinical stage is assigned according to algorithms defined by Rai et al. (1975) or Binet et al. (1981), using physical findings and the results of a complete blood count. These time-honored approaches remain the mainstays of clinical care.

Predictions of clinical course and outcome can be made by analyzing a series of laboratory

parameters. Perhaps the most reliable prognostic indicator is “IGHV mutation status”: the presence or absence of significant numbers of somatic mutations in the *IGHV* gene expressed by the CLL clone. As discussed, CLL clones can be divided into two categories, based on mutations in the heavy chain variable gene, *IGHV*-unmutated (“U-CLL”; $\leq 2\%$ difference from the most similar germline gene) and *IGHV*-mutated (“M-CLL”; $> 2\%$ difference from the most similar germline gene) (Schroeder and Dighiero 1994; Hashimoto et al. 1995; Fais et al. 1998), with patients bearing a U-CLL clone having a more aggressive clinical course (Damle et al. 1999; Hamblin et al. 1999).

Other cell-based indicators suggesting an inferior clinical course include increased percentages of CLL cells bearing membrane CD38 (Damle et al. 1999) or CD49d (Shanafelt et al. 2008a; Bulian et al. 2014; Baumann et al. 2016) or intracellular levels of ZAP70 (Crespo et al. 2003; Rassenti et al. 2008). Furthermore, β_2 -microglobulin (Simonsson et al. 1980; Di Giovanni et al. 1989; Keating et al. 1995) and thymidine kinase (Hallek et al. 1999) serum levels provide prognostic information; the former is easier to analyze and is preferred in clinical settings.

Finally, serial absolute lymphocyte counts afford helpful information. Patients whose absolute lymphocyte counts double in < 1 yr are more likely to follow an aggressive course (Montserrat et al. 1986; Molica and Alberti 1987).

Predictive Markers

Whereas prognostic markers try to presage the likelihood of disease progression and hence clinical outcome, predictive markers try to foresee the response to a given therapy (Oldenhuis et al. 2008; Mandrekar and Sargent 2010; Zenz et al. 2010b; Montserrat 2012). Because CLL is a heterogeneous disease at the clinical and molecular levels, it is difficult to define parameters that best predict outcome to a specific therapy. Moreover, because the therapies for CLL are currently under major flux, the principles defined for one approach might not apply to another. With these caveats in mind, genetic abnormalities,

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particularly loss or mutation of *TP53*, are predictive for certain therapies. Patients with *TP53* disruption do not respond to chemoimmunotherapy and might be compromised by it (Gonzalez et al. 2011). Moreover, although patients with this genetic lesion respond to signaling inhibitors better than to chemoimmunotherapy, these patients are more likely to relapse (Byrd et al. 2015; Farooqui et al. 2015). Additionally, the presence of mutations in *NOTCH1* can signal ineffectiveness of anti-CD20 mAb treatment because of diminished numbers of membrane CD20 molecules (Pozzo et al. 2016).

Possibly the most reliable predictive indicator for patients treated with chemotherapy and chemoimmunotherapy is the level of minimal residual disease (MRD) detectable after therapy. MRD is evaluated using multiparameter flow cytometry (Rawstron et al. 2013; Böttcher 2019), using polymerase chain reaction detection of the signature VH CDR3 segment of the CLL clone (van Dongen et al. 2003), and, recently, by next-generation deep sequencing of the CLL *IGHV-IGHD-IGHJ* rearrangement (Logan et al. 2011, 2013; Rawstron et al. 2015; Rodríguez-Vicente et al. 2017).

The extent of MRD independently predicts progression-free survival and overall survival in patients receiving various chemotherapy and chemoimmunotherapy regimens in the front-line, follow-up, and consolidation settings (Böttcher et al. 2012; Kwok et al. 2016; Varghese et al. 2017). Hence, MRD can be used as a surrogate for progression-free survival in therapeutic clinical trials (Dimier et al. 2018). However, a role for MRD in patients treated with the signaling pathway inhibitors or anti-BCL2 therapy is not clear, especially for the former because most patients receiving these medications continue to have detectable leukemic cells.

Combinatorial Indices

Because of the complexity and variability in clinical course and in leukemia-cell biology in individual patients, combinatorial algorithms incorporating several prognostic and predictive markers have been devised to better predict outcome and response to therapy (Wierda et

al. 2007, 2011; Molica et al. 2010; Rossi et al. 2013).

The Chronic Lymphocytic Leukemia International Prognostic Index (CLL-IPI) incorporates patient age, disease stage (Rai or Binet systems), *TP53* disruption, *IGHV* mutation status, and serum β 2-microglobulin levels (International CLL-IPI Working Group 2016). These five parameters are weighted individually, and a composite score assigns patients to one of four risk groups. Another algorithm using only *IGHV* mutations and high-risk fluorescence in situ hybridization (FISH) cytogenetics (*del17p* and *del11q*) divides CLL patients into three groups: low-risk, comprised of M-CLL clones without high-risk cytogenetics; intermediate-risk, containing either U-CLL or high-risk cytogenetics; and high-risk, consisting of U-CLL and high-risk cytogenetics (Delgado et al. 2017). Time to first treatment and overall survival follow the order of the risk categories for both approaches. Should the same conclusions be drawn for treatments with the signaling inhibitors and the BCL-2 family blocker (Molica et al. 2018), these might eventually be useful in daily practice and to stratify patients in clinical trials.

Finally, despite the information that prognostic, predictive, and combination indicators suggest, the decision to begin therapy in clinical practice is still based on an educated synthesis of patient information and symptoms, physical examination findings, and laboratory data to define active disease (Hallek et al. 2018).

Options for the Initial Treatment of CLL

A physician treating CLL patients in 2019 has many previously unavailable options. Because most CLL patients are aging and might have compromised organ systems that reduce treatment tolerability, patient fitness and comorbidities influence therapy goals (complete remission vs. symptom alleviation/palliation). Table 3 provides therapeutic options for patients in various fitness and disease categories. At this point, the most frequently employed are chemoimmunotherapy, mAbs to B-cell surface molecules, and inhibitors of signaling and anti-apoptotic pathways.

Table 3. Therapeutic options for chronic lymphocytic leukemia (CLL) patients in various fitness and disease categories for front-line and advanced disease settings

Co-morbidities	CLL cell genetic profile	IGHV mutation status	IGHV			Suggested therapy for relapsed/refractory patients	Therapeutic goals for secondary therapies
			Suggested front-line therapy	Therapeutic goals for front-line therapy			
None/mild	Absence of del17p/TP53 mutation	M-CLL	FCR < 65 yr	Long-term remission with prolonged survival	Ibrutinib	Ibrutinib Idelalisib + Ritux Venetoclax Obinu + CLB Duvelisib	Long-term remission
			Ibrutinib		Obinu + CLB		
			Obinu + CLB		Ibrutinib		
	Presence of del17p/TP53 mutation	Not relevant	Ibrutinib	Long-term remission with prolonged survival	Obinu + CLB	Venetoclax + Ritux	Long-term remission
Significant	Absence of del17p/TP53 mutation	Not relevant	Ibrutinib	Disease control and improved quality of life	Ibrutinib	HSC transplantation Ibrutinib Idelalisib + Ritux Obinu + CLB Venetoclax	Disease control
			Obinu + CLB		Ibrutinib		
			BR		Ibrutinib		
	Presence of del17p/TP53 mutation	Not relevant	Ibrutinib	Disease control and improved quality of life	Ibrutinib	Venetoclax	Disease control
Overriding	Irrelevant	Irrelevant	Ibrutinib	Disease control and improved quality of life Palliation	Ibrutinib	Duvelisib Ibrutinib Venetoclax Ibrutinib Idelalisib + Ritux Venetoclax Obinu + CLB	Disease control Palliation
			Obinu + CLB		Ibrutinib		
			Ritux ± CLB		Ibrutinib		
	Irrelevant	Irrelevant	Ofa ± CLB	Palliation	Ibrutinib	Obinu + CLB	Palliation

FCR, Fludarabine/cyclophosphamide/rituximab; Obinu, obinutuzumab; CLB, chlorambucil; Ritux, rituximab; Ofa, ofatumumab; BR, bendamustine/rituximab; M-CLL, IGHV-mutated CLL; U-CLL, IGHV-unmutated CLL.

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Chemoimmunotherapy

Historically, chemotherapy has been the mainstay of cancer therapy, with addition of mAbs leading to improved regimens. In CLL, fludarabine plus cyclophosphamide (FC) were synergistic (Keating et al. 1989; Yamauchi et al. 2001); adding rituximab (FCR) resulted in an even more effective regimen (Keating et al. 2005; Tam et al. 2008; Hallek et al. 2010; Fischer et al. 2016; Thompson et al. 2016). Presently, FCR is considered state-of-the-art therapy for physically fit CLL patients. Remarkably, ~50% of previously untreated M-CLL patients are alive without progression at ~13 yr, compared to only <10% of U-CLL patients (Thompson et al. 2016), suggesting this treatment may cure some M-CLL patients. Studies with shorter follow-up times (Fischer et al. 2016) and fewer cases (Rossi et al. 2015) report similar results, although high serum β_2 -microglobulin levels and del17p and/or del11q herald less durable remissions (Rossi et al. 2015). Bone marrow (BM) and immune suppression following this regimen (Benjamini et al. 2015), however, raise concerns about secondary malignancies, especially in patients receiving FCR as frontline therapy (Zhou et al. 2012; Benjamini et al. 2015; Falchi et al. 2016).

Monoclonal Antibodies

Three anti-CD20 mAbs, classified as Type I or II based on binding properties (Beers et al. 2010), are approved for use in CLL. Type I mAbs (rituximab and ofatumumab) effectively promote cytotoxicity but are less effective at inducing apoptosis (Cragg et al. 2003), whereas type II (obinutuzumab) is superior at inducing apoptosis but inferior at carrying out cytotoxicity (Chan et al. 2003). In previously untreated CLL patients, type II obinutuzumab, in combination with chlorambucil, is superior to type I rituximab plus chlorambucil (Goede et al. 2014).

Specific Pathway Inhibition

Studies associating unique structural BCR features with clinical outcome and differences in signaling capacities brought small molecules

blocking enzymes critical for this signaling pathway into the clinic. Orally administered inhibitors of Bruton's tyrosine kinase (Btk), ibrutinib, and of phosphatidylinositol-4,5-bisphosphate 3-kinase delta (PI3K δ), idelalisib, yielded dramatic responses in relapsed/refractory patients by blocking signaling through the BCR, chemokine receptors, and integrins (Figs. 2 and 3; Spaargaren et al. 2003; Ortolano et al. 2006; de Gorter et al. 2007; de Rooij et al. 2012; Herman et al. 2015; Maffei et al. 2015; Chen et al. 2016). Ibrutinib is efficacious in patients with del17p, del11q, and unmutated *IGHV* (Farooqui et al. 2015) and in patients refractory or unresponsive to other therapies (Byrd et al. 2013; Furman et al. 2014b). Both molecules inhibit cell replication (Herman et al. 2011, 2014a; Ponader et al. 2012), trafficking to/retention in lymphoid tissues (Niedermeier et al. 2009; Herman et al. 2011, 2014a,b; Ponader et al. 2012; Fiorcari et al. 2013; Göckeritz et al. 2015), and production of pro-inflammatory cytokines (Ponader et al. 2012; Herman et al. 2014a; Niemann et al. 2016). Upon initiating ibrutinib or idelalisib therapy, lymphadenopathy and splenomegaly rapidly regress and peripheral blood lymphocyte counts increase because of redistribution of cells from tissue compartments to the blood (Woyach et al. 2014b; Chen et al. 2016; Burger et al. 2017). In most patients, lymphocytosis diminishes over time and circulating B-cell numbers in the blood fall to or below pretreatment levels. Ibrutinib is FDA-approved for first- and second-line therapy of CLL and for patients with del17p; idelalisib in combination with rituximab is approved for relapsed CLL. Recently, an inhibitor of both PI3K δ and PI3K γ (duvelisib) was approved for the treatment of relapsed/refractory CLL and SLL (Flinn et al. 2018).

As single agents, these medications rarely lead to complete remissions (Burger et al. 2010). Also, occurrence of long-term side effects and development of resistance have not been fully investigated. Ibrutinib and idelalisib are generally well-tolerated, and severe side effects occur in a minority of patients (de Weerd et al. 2017). For ibrutinib, these are bleeding (Lipsky et al. 2015), hypertension (Caldeira et al. 2019), and atrial fibrillation (McMullen et al. 2014;

Lentz et al. 2019). For idelalisib, neutropenia and inflammatory reactions (Brown 2014; Furman et al. 2014b), particularly in treatment-naïve patients (Lampson et al. 2016), are major adverse events, and hence idelalisib plus rituximab is reserved for relapsed, refractory disease.

Targeting Anti-Apoptotic Proteins

CLL lymphocytes characteristically overexpress anti-apoptotic BCL-2 (Schena et al. 1992), the parent molecule in a family of proteins controlling suppression or initiation of apoptosis (Adams and Cory 2018). BCL-2 family members containing BCL-2 homology domain-3 (BH3-only proteins) promote apoptosis by blocking pro-survival members of the family (Ruefli-Brasse and Reed 2017). Venetoclax, an orally administered BH3-protein mimetic selectively targeting BCL-2 (Souers et al. 2013), is effective in refractory CLL patients with del17p, del11q, or *IGHV*-unmutated clones (Anderson et al. 2016) and is FDA-approved for previously treated CLL patients bearing del17p clones. Overall remissions and complete remissions have been achieved in 82% and 10% of patients, respectively, and in 5%, residual disease was not detectable at 15 mo (Roberts et al. 2016). Because of the propensity to cause tumor lysis syndrome, patients are started on very low drug doses initially.

DISEASE PROGRESSION AND THERAPEUTIC REFRACTORINESS TO INITIAL THERAPY

Factors Promoting Development of More Aggressive Clonal Variants

Like other cancers, progression of CLL develops from the emergence of intraclonal variants with genetic changes fostering growth, survival, and therapeutic resistance. These disrupt the balance between cellular proliferation and death (Chiorazzi 2007) and in some patients become a major component of the leukemic clone. Such variants can develop spontaneously or are generated by processes inherent to normal cells or unique to a cancer cell; certain treatments targeting replicating DNA can do the same.

CLL cells contain high levels of reactive oxygen species (Oltra et al. 2001; Zhou et al. 2003), a characteristic of cancer cells, which can act as mutagens (Cerutti 1994; Wiseman and Halliwell 1996; Nogueira and Hay 2013). Also, as B lymphocytes, CLL cells can make the mutagenic enzyme, AID (Albesiano et al. 2003; McCarthy et al. 2003; Oppezzo et al. 2003). AID carries out a normal role in the development of more effective immune responses by inducing mutations in genes coding the variable domains of Abs/IGs (*IGHV*, *IGHD*, *IGHJ* and *IGLV*, *IGLJ*) and in the gene segments that support switching from IgM to IgG, IgA, and IgE (Muramatsu et al. 1999, 2000). However, AID can act aberrantly, creating mutations outside IG loci (Pasqualucci et al. 1998; Yamane et al. 2011), leading to genetic changes and B-cell lymphomas (Pasqualucci et al. 2001; Lenz et al. 2007; Robbiani et al. 2009).

A small fraction (~0.01%–1%) of circulating CLL B cells express AID mRNA (Albesiano et al. 2003), and so AID protein is rarely detectable (Pasqualucci et al. 2004). However, CLL cells in tissue proliferation centers (Leunberger et al. 2010; Patten et al. 2012) and those activated in vitro (Cerutti et al. 2002; Albesiano et al. 2003; Oppezzo et al. 2003) and growing in xenografts (Patten et al. 2016) synthesize AID protein, consistent with the link of AID production to cell division (Rush et al. 2005). The protein is functional as evidenced by detection of new, spontaneous mutations in the clonal *IGHV-IGHD-IGHJ* (Patten et al. 2012, 2016) and hence could create off-target mutations and intraclonal variants that might lead to disease progression. Although certain chemotherapeutic agents can induce DNA mutations (Tan et al. 2015), based on next-generation deep-sequencing analyses many/most mutations that emerge after cancer therapy in CLL patients are present before treatment. Mathematical modeling suggests this is likely for ibrutinib (Komarova et al. 2014). Also, because the drug prevents CLL B-cell division (Honigberg et al. 2010; Herman et al. 2011; Burger et al. 2017) and AID is produced upon cell proliferation (Rush et al. 2005), it is likely that additional clonal mutations are halted by ibrutinib.

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Clonal Evolution

The common genetic abnormalities identified by FISH and the recurrent genetic mutations defined by NGS (Table 1) have been used to follow clonal evolution in patients (Finn et al. 1998; Shanafelt et al. 2008b; Landau et al. 2013, 2014a, 2015; Lawrence et al. 2013; Nadeu et al. 2018). Similarly, epigenetic diversity can identify similar events (Kulis et al. 2012; Oakes et al. 2014; Landau et al. 2014b; Guièze and Wu 2015; Queirós et al. 2015; Mansouri et al. 2018). Resulting clonal architectures can be informative (Messina et al. 2014; Wang et al. 2014; Ojha et al. 2015). Whereas linear development from a starting genetic template is seen more often, branching patterns are also found (Braggio et al. 2012), indicating distinct intraclonal variants can evolve in parallel. These chronologic genetic and epigenetic variations provide insights into disease progression and into the clinical courses for individual patients (Quesada et al. 2013; Villamor et al. 2013; Rose-Zerilli et al. 2016).

Role of CLL-Cell Growth Rate in Disease Progression

CLL progresses at a slower rate than acute leukemias and more aggressive lymphomas, as reflected by clonal birth rates and by the relatively low baseline levels of genomic mutations. Approximately 0.1%–2% of a CLL clone is added daily, based on deuterium (^2H)-labeling of replicating DNA provided as “heavy water” ($^2\text{H}_2\text{O}$) to measure CLL B-cell growth in patients (Messmer et al. 2005; Defoiche et al. 2008; van Gent et al. 2008). Clonal birth rates directly correlate with clinical course, as patients with higher birth rates require treatment sooner (Murphy et al. 2017). Also, most CLL-cell growth occurs in lymph nodes (LNs) (Herndon et al. 2017), suggesting CLL cells divide in secondary lymphoid tissues and traffic to the BM, where they reside mostly in a resting state (Fig. 4; Calissano et al. 2011). This model for growth and survival based on tissue residence has been recapitulated in murine models (Chen et al. 2013). Hence, CLL cells at different anatomic sites can behave differently, which may relate to effectiveness of certain therapeutic agents.

Relapsed/Refractory, Accelerated, and Transformed Disease

Here we assume that first-line treatments mentioned in Table 1 have failed, and the patient again requires therapy. Consistent with clonal evolution and disease progression being a function of increased cell growth in LNs, patients in this category often have nodal, splenic, or extranodal disease. A suspicion of Richter’s transformation (RT) (Richter 1928), often as diffuse large B-cell lymphoma (DLBCL) that has large B cells with nuclear sizes more than twice that of a normal B lymphocyte, is raised when LN or spleen enlargement occurs rapidly and when extranodal sites are defined; elevated LDH levels are often found (Jain and Young 2014; Allan and Furman 2019). In those instances in which RT is not found, patients might have “accelerated CLL,” defined by highly proliferative CLL cells in expanded, substantially more active proliferation centers that contain T cells, especially CD4^+ cells, and dendritic cells. Although the frequencies of accelerated CLL and RT are only ~23% and ~10% of all CLLs, they are increased for those relapsed/refractory patients with adenopathy (~25% accelerated CLL, ~50% standard refractory CLL, and ~25% RT) (Gine et al. 2010). Survival in the three categories are distinct: ~76 mo in nonaccelerated CLL, 34 mo for accelerated CLL, and only 8–12 mo for RT.

The mechanisms leading to accelerated CLL and RT are unclear, although they might involve BCR and TLR signaling to stimulate CLL-cell proliferation (Balogh et al. 2011), especially stereotyped subset #8 CLLs (Rossi et al. 2009b) that display polyreactivity to multiple antigens (Gounari et al. 2015). The lymphoma that develops in RT is usually clonally related to the CLL clone (Mao et al. 2007), and when that is the case the outcome for the patient is worse than if the lymphoma has a distinct genetic origin (Condoluci and Rossi 2017). Several genetic alterations are associated with RT: elevated AID levels in leukemic B cells (Reiniger et al. 2006), disruption of TP53 and CDKN2A, and activation of NOTCH1 and c-MYC (Fabbri et al. 2013). TP53 and CDKN2A mutations are found in >50% of cases, whereas trisomy 12 and

Figure 4. Life cycle of a chronic lymphocytic leukemia (CLL) B lymphocyte. In primary and secondary lymphoid tissues (*bottom left*) CLL B lymphocytes nestle, in a resting state, on survival-nurturing cells (e.g., nurse-like cells [Burger et al. 2000] or less well-defined, nonhematopoietic “stromal cells”). Docking is facilitated by several receptor–ligand interactions, including CXCL12–CXCR4. When cell division initiates, spontaneously or after stimulation via receptors (e.g., B-cell receptor [BCR] or Toll-like receptors [TLRs]), cells internalize CXCR4, detach, and migrate to structures resembling germinal centers (“proliferation centers” [Swerdlow et al. 1984; Schmid and Isaacson 1994; Bonato et al. 1998]), sites of CLL B-cell expansion. Activated/dividing cells up-regulate a number of surface proteins that promote interactions with T lymphocytes. These interactions up-regulate the DNA-mutating enzyme AID, which can cause mutations in genes genome-wide, leading to clonal evolution and possibly more aggressive disease. After expansion, some recently divided CLL cells exit the lymphoid tissue, entering the circulation bearing the CXCR4^{Dim}CD5^{Bright} phenotype (“proliferative fraction” [Calissano et al. 2011]). Over time, the circulating cells express more CXCR4 and less CD5, eventually morphing into a CXCR4^{Bright}CD5^{Dim} resting fraction (RF) phenotype. These cells are best suited to follow a CXCL12/SDF1 gradient because of high levels of CXCR4, returning back to nutrient-rich niches in tissues and being rescued from apoptosis by IL-4 and likely other cytokines. Cells that cannot reenter tissues or do not arrive soon enough die. Once rescued, CLL cells proceed to a proliferation center to re-initiate the proliferative process and potential further clonal evolution or dock on a stromal element, where they again reside in the resting state.

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NOTCH1 mutations are found in >30% of cases (Allan and Furman 2019). A microRNA expression signature has also been identified in RT (Van Roosbroeck et al. 2018).

Therapy for Relapsed/Refractory and Accelerated Disease and for Richter's Transformation

Table 3 lists a series of therapies for patients in these categories, again based on the physical condition of the patient and the goal of treatment. For patients relapsing on ibrutinib, progression appears accelerated and less well-controlled (Jain et al. 2015; Maddocks et al. 2015). Leukemic cells of patients failing ibrutinib can contain mutations at the site in the Btk molecule where the drug binds (cysteine 481) or in downstream signaling molecules (Furman et al. 2014a; Woyach et al. 2014a; Cheng et al. 2015); such variants appear to be selected from cells bearing these mutations existing prior to treatment (Woyach et al. 2014a).

Outcomes for RT patients treated with chemoimmunotherapy remain dismal. Because of the ineffectiveness of chemoimmunotherapy and the dysfunction of T cells, NK cells, and other immune subsets, novel agents targeting RT cells and their interaction with immune subsets in LNs are being developed.

In a few reports, treatment with ibrutinib induced partial responses or retained stable disease in RT patients (Giri et al. 2015; Fischer et al. 2018); this could be caused by reduced proliferation of CLL/RS cells expressing AID (Reiniger et al. 2006). However, CLL patients that develop accelerated CLL or RT can progress on ibrutinib (Kadri et al. 2017); because ibrutinib is now used as front-line therapy, finding an effective therapy for this subset of patients is urgently needed. The BCL-2 inhibitor, venetoclax, alone (Jones et al. 2018) or in combination with ibrutinib (Flinn et al. 2019; Rogers 2019) may be beneficial for relapse/refractory patients and for RT (Roberts et al. 2016), although the effectiveness of these and resistance to venetoclax (Blombery et al. 2019) are evolving.

T-cell exhaustion and impaired T cell–B cell interactions promote disease progression in

patients with RT. Improving T-cell function with anti-PD1 or anti-PDL1 mAb treatments (pembrolizumab or nivolumab) was therapeutic in a CLL mouse model (McClanahan et al. 2015), in patients with relapsed/refractory Hodgkin lymphoma (Ansell et al. 2015), and in patients with CLL-derived RT (Ding et al. 2015). The combination of pembrolizumab and ibrutinib in patients with RT is being evaluated (NCT20332980). Overall, BCR inhibitors and immune checkpoint inhibitors appear promising, although the number of patients treated is limited, and studies of larger patient cohorts are needed to define optimal therapeutic strategies.

An approach to enhancing actions of cytolytic T lymphocytes comes from work inserting, into the T-cell surface membrane, the antigen-binding portion of an antibody that binds B lymphocytes (e.g., CD19) linked to the constant and signaling regions of a T-cell receptor (Gross et al. 1989; Eshhar et al. 1993). These engineered CAR-T cells kill target cells independent of histocompatibility constraints and the requirements for antibody-dependent and complement-dependent cytotoxicity.

CAR-T therapy has had dramatic results in selected relapsed/refractory CLL patients (Brentjens et al. 2011; Porter et al. 2011), and larger studies indicate beneficial responses in ~30%–50% of patients (Frey and Porter 2016; Romero 2017; Turtle et al. 2017). Using T cells from patients who received ibrutinib (Fraietta et al. 2016; Harper 2019) or cells expanded in the presence of PI3K δ inhibitors (Petersen et al. 2018) or the selective expansion of autologous CD4⁺ and CD8⁺ cells infused at defined CD4 to CD8 ratios (Sommermeyer et al. 2016; Turtle et al. 2016) have improved effectiveness.

However, CAR-T cells can result in major complications. A cytokine release syndrome (Lee et al. 2014) from liberation of pro-inflammatory cytokines (Kochenderfer et al. 2012), including IL-6 (Lee et al. 2014), can occur. Blocking the IL-6 receptor with mAbs is helpful (Lee et al. 2014). The use of CAR-T therapy will increase as effective ways of lessening complications emerge (Acharya et al. 2019). Moreover, because CAR-Ts use anti-B-cell antibodies to

target CLL cells, normal B lymphocytes are also eliminated, leading to hypogammaglobulinemia (Kochenderfer et al. 2010).

Implications for More Effective Disease Interventions and Possible Cures

Several avenues could lead to new therapies and hopefully cures. Considering the major beneficial impact inhibitors of BTK and PI3K signaling have had on CLL, it is likely that combinations of these drugs with newly approved agents such as venetoclax will lead to more profound, long-lasting effects and longer overall survival; several such studies are under way. Moreover, targeting other signaling pathways supporting survival and growth of CLL cells may be valuable adjuncts to current enzyme inhibitors.

As deep DNA sequencing becomes more available, rapid, and inexpensive, therapeutic decisions might take into consideration specific gene abnormalities and clonal architecture in individual patients. Personalized therapy might involve replacing/overexpressing/removing microRNAs because they can be oncogenic or tumor-inhibitory. Similarly, targeting patient-specific driver mutations and the pathways they derange, early or during disease progression, could achieve this goal.

Because of their shared features, targeting stereotyped BCRs might be feasible for discrete groups of patients. Development of small molecules reactive with stereotypes (Liu et al. 2013; Sarkar et al. 2014, 2016) and humanized mAbs binding specific *IGHV*s (Chang et al. 2016) support this possibility.

Finally, safer and more specific and effective CAR-T cells, and the reinfusion of functionally competent autologous T cells capable of recognizing CLL-specific antigens, similar to those emerging in myeloma (Thompson et al. 2003), may advance cellular therapy.

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