

Review Article

# Pathogenesis of chronic lymphocytic leukemia and the development of novel therapeutic strategies

Yoshikane Kikushige<sup>1,2)</sup>

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in Western countries and is characterized by the clonal expansion of mature CD5<sup>+</sup> B cells. There have been substantial advances in the field of CLL research in the last decade, including the identification of recurrent mutations, and clarification of clonal architectures, signaling molecules, and the multistep leukemogenic process, providing a comprehensive understanding of CLL pathogenesis. Furthermore, the development of therapeutic approaches, especially that of molecular target therapies against CLL, has markedly improved the standard of care for CLL. This review focuses on the recent insights made in CLL leukemogenesis and the development of novel therapeutic strategies.

**Keywords:** chronic lymphocytic leukemia, multistep leukemogenesis, BCR signaling, novel drugs

## INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia. It is characterized by the clonal expansion of CD5<sup>+</sup> mature B cells in the blood, bone marrow, and lymphoid tissues.<sup>1</sup> CLL typically affects elderly people.<sup>2</sup> Recent advances in next-generation sequencing (NGS) technologies revealed recurrent somatic mutations and identified the molecular pathways involved in CLL pathogenesis. Furthermore, the analysis of clonal architecture clarified that CLL genomes exhibit heterogeneity between patients with CLL and within cells of individual patients.<sup>3</sup> Such advances in genetic lesion analysis significantly improved our understanding of the leukemogenic process of CLL. Moreover, CLL leukemogenesis has been described as a multistep process originating from immature hematopoietic stem cells (HSCs).<sup>4,5</sup> Thus, our understanding of CLL biology has significantly improved in the last decade. The clinical efficacy of novel drugs against CLL, such as the tyrosine kinase inhibitor ibrutinib and the B cell lymphoma 2 (BCL2) antagonist venetoclax, has markedly improved the standard of care for specific subsets of patients with CLL. This review focuses on recent insights into CLL leukemogenesis, emphasizing the immunological aspects of B cell receptors (BCRs), genetic lesions, and the process of multistep CLL leukemogenesis. It also describes the advances in the field of basic

CLL research and the development of novel therapeutic strategies against CLL.

## BIOLOGICAL AND IMMUNOLOGICAL FEATURES OF CLL

CLL is a B cell malignancy characterized by accumulating mature clonal CD5-expressing B cells.<sup>6-8</sup> The CLL prevalence markedly increases with age. CLL cells express functional BCRs on their surfaces.<sup>9,10</sup> CLL is classified into two subgroups based on the presence of somatic hypermutations within the variable regions of the immunoglobulin heavy chain gene (*IGHV*). Patients with CLL with mutated *IGHV* (*IGHV*-M CLL) have a more favorable prognosis than those with unmutated *IGHV* (*IGHV*-UM CLL).<sup>11</sup> The somatic hypermutations occur in the process of normal B cell development in the germinal centers during the naïve-to-memory B cell transition. Initial studies proposed distinct origins of the two types of CLL, with *IGHV*-UM CLL originating from naïve B cells and *IGHV*-M CLL originating from antigen-experienced B cells, including memory B cells. However, immunological analysis of CLL-BCRs revealed that both types recognized self-antigens, at least *in vitro*, suggesting that CLL originates from self-reactive B cell precursors, irrespective of the *IGHV* mutation status.<sup>12,13</sup> The self-reactivity of BCRs from CLL is one of the most extensively investigated


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<sup>1)</sup>Department of Medicine and Biosystemic Sciences, Kyushu University Graduate School of Medicine, Fukuoka, Japan, <sup>2)</sup>Center for Cellular and Molecular Medicine, Kyushu University Hospital, Fukuoka, Japan

**Corresponding author:** Yoshikane Kikushige, Department of Medicine and Biosystemic Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-Ku, Fukuoka 812-8582, Japan.  
E-mail: kikushige@intmed1.med.kyushu-u.ac.jp

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biological features of human CLL. BCR signaling is constitutively activated and is an important biological feature of CLL cells,<sup>14</sup> providing rationale for BCR signaling-targeted therapies for CLL. Of note, the BCRs of ~1% of CLL cases express a nearly identical amino acid sequence of heavy chain complementarity-determining region 3 (HCDR3), and the BCRs of CLL cells can be classified as specific stereotyped BCRs in ~30% of CLL cases.<sup>15-17</sup> These observations led to the hypothesis that some common self-antigens recognized by CLL-BCRs drive clonal expansion and play a significant role in CLL pathogenesis. Consistent with this hypothesis, recent studies successfully identified common auto- or exoantigens, including myosin heavy chain IIA,<sup>18</sup>  $\beta$ -(1,6)-glucan,<sup>19</sup> and rheumatoid factors (RFs).<sup>20,21</sup> Such antigens are considered drivers of the expansion of CLL cells via BCR signaling.

In addition to the antigen-dependent mechanisms of CLL-BCR activation, two recent studies clarified the antigen-independent BCR activation mechanism in CLL. Duhren-von Minden *et al.* revealed that HCDR3 of CLL-BCRs recognizes the specific amino acids of the second framework region of immunoglobulins (Igs), inducing  $Ca^{2+}$  signaling independent of specific antigens.<sup>22</sup> Consistent with this theory, Binder *et al.* identified the alternative epitope recognized by CLL-BCRs in the third framework region of Igs.<sup>23</sup> Thus, CLL-BCRs have unique HCDR3 rearrangements that ensure basal BCR signaling activity via the self-recognition of CLL-BCRs. This characteristic may be specific to CLL-BCRs because BCRs from other B cell malignancies lack the self-recognition of Igs.<sup>22</sup> Furthermore, it is consistent with CLL-BCRs being able to bind the Igs component, acting like RFs.<sup>20,21,24,25</sup> Collectively, these studies suggest the presence of antigen-dependent and -independent mechanisms for the constitutive activation of BCR signaling in CLL.

## CLL INTERACTION WITH THE MICROENVIRONMENT

In addition to cell-intrinsic molecular mechanisms that regulate the survival and proliferation of CLL cells, the interaction between CLL cells and their microenvironment also plays an essential role in CLL progression.<sup>26,27</sup> CLL cells recirculate between peripheral blood and secondary lymphoid tissues, where CLL cells proliferate at a daily birth rate of 0.1%–2.0% of all CLL clones in humans.<sup>28</sup> The lymphoid tissues of CLL exhibit unique histopathological features termed proliferation centers or pseudofollicles.<sup>29</sup> The homing process to such lymphoid tissues is essential for CLL propagation and is tightly regulated by cytokines, chemokines, chemokine receptors, and adhesion molecules. CLL cells secrete several cytokines that attract accessory cells, such as T cells and monocytes/macrophages, thereby altering their anti-leukemic activity.<sup>30-33</sup> T cells from CLL patients exhibit features of exhaustion but retain their capacity for cytokine production,<sup>34</sup> and such T cells demonstrate impaired immunological synapse formation.<sup>35</sup> CLL cells alter T cell gene expression via direct cell–cell contact.<sup>36</sup> Of note,

alterations in T cell subsets, such as helper T cells and Th17 cells, have been reported in CLL patients with autoimmune cytopenia.<sup>37</sup>

Nurse-like cells (NLCs) represent an important component of the CLL microenvironment. They are derived from monocytic cells and are found in the lymphoid organs of CLL patients.<sup>38,39</sup> NLCs exhibit gene expression patterns similar to M2 macrophages and tumor-associated macrophages,<sup>40,41</sup> which support the propagation of solid tumors. Of note, CLL cells produce extracellular nicotinamide phosphoribosyltransferase, which is involved in the induction of monocyte polarization to M2 macrophages secreting tumor cytokines and inhibiting T cell response.<sup>42</sup> Gene expression analysis revealed that the interaction between CLL cells and NLCs activated BCR and NF- $\kappa$ B signaling pathways in CLL.<sup>43,44</sup> Consistent with the alteration of gene expression signatures, CLL-BCR recognizes some antigens, such as vimentin and calreticulin, that are highly expressed on NLCs.<sup>45</sup> Thus, the interaction between CLL cells and NLCs is one of the CLL-specific mechanisms driving BCR signaling in CLL.

In addition to hematopoietic cells, mesenchymal stromal cells are also involved in CLL pathogenesis as one of the essential components of the CLL-specific microenvironment. Bone marrow stromal cells (BMSCs) support CLL cell survival through direct cellular interaction,<sup>46</sup> and protect CLL cells from spontaneous and drug-induced apoptosis.<sup>47</sup> Similar to NLCs, direct cellular interactions between CLL cells and BMSCs induce BMSC activation via the protein kinase C (AKT)- $\beta$ II/NF- $\kappa$ B signaling pathway.<sup>48</sup> Furthermore, CLL cells release microvesicles carrying signaling molecules that activate the AKT pathway in BMSCs.<sup>49,50</sup> Thus, both direct and indirect interactions result in bidirectional crosstalk between CLL cells and BMSCs. These studies highlight the significance of the microenvironment in CLL pathogenesis. Thus, further studies targeting the CLL-specific microenvironment will aid in the development of novel therapeutic strategies.

## GENETIC AND EPIGENETIC CHARACTERISTICS OF CLL

Although the majority of CLL cases develop sporadically, an inherited predisposition to CLL has been reported.<sup>51</sup> Relatives of CLL patients have an increased risk of CLL and non-Hodgkin's lymphoma.<sup>52</sup> Furthermore, the incidence of CLL is highest in Europe and individuals of European descent worldwide, whereas the lowest incidence is in East Asia, including Japan. Furthermore, a study of migrants revealed a low incidence of CLL in Asian individuals born in the United States.<sup>53</sup> These observations suggest the presence of a genetically inherited predisposition to CLL. Several genome-wide association studies (GWASs) identified multiple risk loci for CLL;<sup>54-58</sup> however, the statistical power of individual GWASs was limited due to the modest effect size of each genetic variant.

Chromosomal abnormalities in CLL have been extensively investigated and utilized for risk assessments of CLL,

in addition to the genetic predisposition of CLL revealed by GWAS.<sup>59</sup> CLL cells harbor immunoglobulin heavy chain (IgH)-related translocations at markedly low frequencies compared with other types of mature B cell malignancies. This genetic characteristic distinguishes CLL from other mature B cell malignancies.

The most frequently observed genetic lesions in CLL are 13q14 deletions (del13q14), found in 50%–60% of cases.<sup>59,60</sup> These are mostly monoallelic and more frequently observed in *IGHV*-M CLL cases than in *IGHV*-UM CLL cases. Del13q14 is generally associated with a favorable prognosis, but the clinical course of CLL is accelerated in patients with large 13q14 deletions involving the retinoblastoma gene (*RBI*).<sup>61</sup> The long non-coding RNAs DLEU2 and DLEU1 and the microRNA clusters miR15A–miR16-1 are found in the minimal deleted region of del13q14.<sup>62–66</sup> Model mice harboring deletions of the corresponding murine locus developed clonal B cell lymphoproliferative disorders, suggesting the significant role of microRNA in CLL pathogenesis.<sup>67</sup> Furthermore, the deletion of microRNAs miR-15A–miR-16-1 resulted in *BCL2* overexpression,<sup>68</sup> providing rationale for CLL therapeutic strategies targeting *BCL2*.

The second common chromosomal abnormality of CLL is deletions in the 11q22–q23 (del11q) chromosomal region, detected in ~15% of cases.<sup>59,60,69</sup> Del11q leads to the loss of tumor suppressor gene ataxia telangiectasia mutated (*ATM*), which encodes the DNA damage response (DDR) kinase ATM.<sup>70</sup> Of note, ~25% of CLL patients with del11q deletions harbor mutations in the remaining *ATM* allele, and the combination of del11q and *ATM* mutation is associated with a poor prognosis.<sup>71</sup>

The third frequently observed chromosomal abnormality is trisomy 12, found in ~15% of patients with CLL.<sup>59,60</sup> Trisomy 12 is a genetic lesion yielding intermediate risk. However, the coexistence of *NOTCH1* mutations and trisomy 12 is associated with poor survival.<sup>72</sup> Moreover, CLL patients with trisomy 12 have a higher risk of transformation into Richter syndrome (RS).<sup>73–75</sup> As RS has a poor clinical prognosis, further studies are recommended to identify the molecular mechanisms by which trisomy 12 increases the risk of RS transformation.

Deletions in the 17p13 chromosomal locus (del17p) are observed in ~10% of patients with CLL<sup>59,60,69</sup> and are more frequently observed in *IGHV*-UM CLL cases than in *IGHV*-M CLL cases.<sup>59</sup> Del17p deletions usually involve the entire short arm of chromosome 17, leading to the loss of the tumor suppressor gene *TP53*.<sup>76</sup> Missense mutations in the remaining *TP53* allele are found in ~80% of patients with CLL and del17p.<sup>77,78</sup> Consistent with the inactivation of *TP53*, patients with del17p exhibit high genomic complexity and a poorer overall prognosis than those with wild-type *TP53*.<sup>59,76–80</sup> Clinically, the assessment of del17p and *TP53* mutation status is essential to select the appropriate therapeutic strategies against CLL.

In addition to the extensive chromosomal abnormalities described above, advances in NGS technologies revealed recurrent driver mutations in CLL such as *SF3B1*, *ATM*,

*TP53*, *NOTCH1*, *POT1*, *CHD2*, *XPO1*, *BIRC3*, *BRAF*, *MYD88*, *EGR2*, *MED12*, *FBXW7*, *ASXL1*, *KRAS*, *NRAS*, *MAP2K1*, *NFKBIE*, *TRAF3*, *RPS15*, and *DDX3X*.<sup>5,60,81–84</sup>

The most frequently mutated gene in CLL is *SF3B1* (10%–15% of cases) and the *SF3B1* K700E mutation is the most common.<sup>81–83</sup> *SF3B1* mutations cause alternative splicing in CLL cells and induce RNA changes affecting multiple CLL-associated pathways, including DDR, telomere maintenance, and NOTCH signaling.<sup>85</sup> In addition to *SF3B1*, several genes involved in RNA splicing, processing, and transport, such as *DDX3X* and *XPO1*, are mutated in CLL at lower frequencies, suggesting that deregulated RNA processing is one of the major pathogenic pathways in CLL development.

*NOTCH1* is the second most commonly mutated gene in CLL (~10% of cases)<sup>60,84,86</sup> and *NOTCH1* mutations are preferentially observed in *IGHV*-UM CLL. Of note, ~40% of patients with *NOTCH1*-mutated CLL harbor trisomy 12, implying a relationship of these two genetic aberrations with CLL development.<sup>72,87</sup> The majority of *NOTCH1* mutations in CLL increase the nuclear NOTCH intracellular domain by abrogating the PEST domain, which is necessary for F-box/WD repeat-containing protein 7-mediated proteasomal degradation of NOTCH1.<sup>3,84,88</sup> *FBXW7*-inactivating mutations have also been observed in ~3% of patients with CLL without *NOTCH1* mutations, demonstrating an analogous outcome of increased NOTCH1 signaling. Moreover, NOTCH1 activation independent of *NOTCH1* mutations has been reported in CLL cells.<sup>89,90</sup> Thus, multiple mechanisms activate the NOTCH1 pathway in CLL pathogenesis.<sup>91</sup>

Somatic mutations affecting inflammation-related genes, such as *MYD88*, *NFKBIE*, *BIRC3*, and *TRAF3*, have been identified as recurrent mutations in CLL.<sup>5,83,92</sup> Mutations in the TLR/MYD88 pathway can be used to identify a subset of young CLL patients with a favorable outcome.<sup>93</sup> Thus, pathological pathways involved in CLL leukemogenesis include inflammatory pathways and their downstream signaling. *POT1* mutations are found in 3%–7% of CLL patients and are frequently observed in *IGHV*-UM CLL.<sup>60,81,82,84,94</sup> *POT1* plays an essential role in telomere protection<sup>95</sup> and is required to maintain the self-renewal capacity of HSCs in normal hematopoiesis.<sup>96</sup> *POT1* mutations in CLL alter the telomeric DNA-binding domain, leading to structural aberrations and chromosomal instability.<sup>94</sup> Thus, the disruption of genes involved in DDR, such as *TP53*, *ATM*, and *POT1*, plays a significant role in CLL development.

In addition to the identification of recurrent mutations in CLL cells, a recent global epigenomic status analysis revealed the regulatory chromatin landscape of CLL, and clarified that *IGHV*-UM CLL cells harbor more active and open chromatin than *IGHV*-M CLL cells. Furthermore, de novo active regions in CLL cells are enriched for NFAT, FOX, and TCF/LEF transcription factors.<sup>97</sup> Of note, most genetic alterations are not associated with specific epigenetic profiles, and CLL with *MYD88* mutations and trisomy 12 exhibit distinct chromatin configurations.<sup>97</sup> In summary, genetic lesions in CLL can be categorized according to several biological pathways such as BCR signaling, inflammatory,

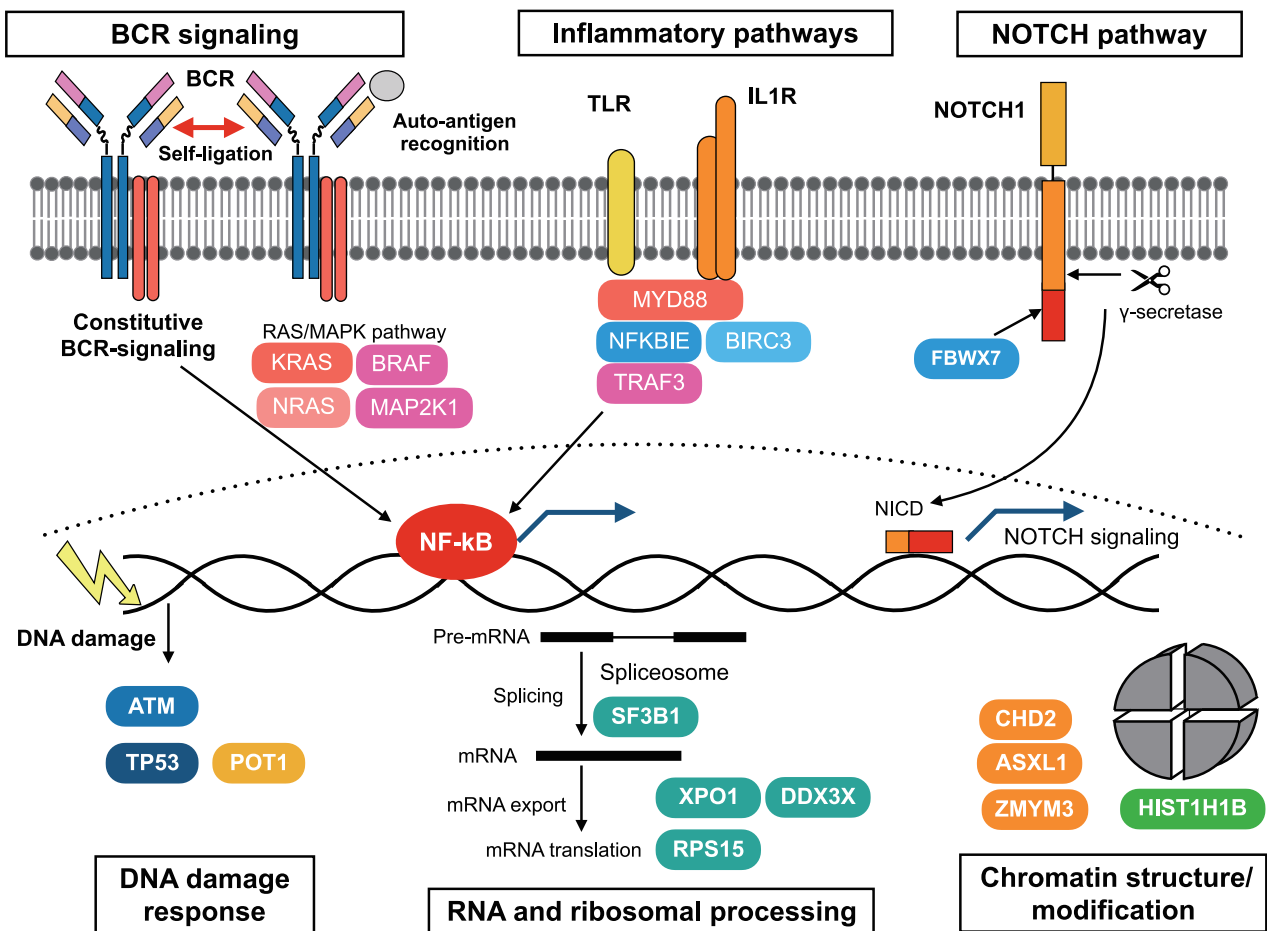
NOTCH1 signaling, DDR, RNA and ribosomal processing, genome/chromatin structure, NF- $\kappa$ B signaling, cell cycle, and apoptosis.<sup>60,84</sup> These deregulated biological pathways coordinately drive CLL leukemogenesis in humans (Figure 1).

**MULTISTEP CLL LEUKEMOGENESIS IS INITIATED BY SELF-RENEWING HUMAN HSCs**

After describing the important molecular pathways identified in NGS studies that are involved in CLL pathogenesis, we next focused on how such oncogenic events initiate and accumulate during leukemogenesis. The pathogenesis of other types of human leukemia, including acute myeloid leukemia, acute lymphoblastic leukemia, and chronic myeloid leukemia, is directly related to HSCs and immature progenitor cells. However, CLL is the exception because it is thought to directly originate from mature B cells. When tracing the origins of human CLL, it must be noted that it is not always monoclonal.<sup>98,99</sup> Moreover, a large cohort study demonstrated that nearly all patients with CLL had prior monoclonal B cell lymphocytosis (MBL),<sup>100</sup> a preleukemic state of CLL with the asymptomatic proliferation of clonal B cells and circulating numbers <5,000/ $\mu$ l.<sup>101</sup> MBL prevalence increases with age,<sup>100,102</sup> ranging from <1%<sup>103,104</sup> to 18%.<sup>105</sup>

Of note, human MBL sometimes comprises oligoclonal B cell clones.<sup>106-110</sup>

Although the progression from MBL to CLL is a stepwise process, the stage at which the first oncogenic event occurs remains unknown. The existence of oligoclonal B cell clones in both patients with CLL and MBL suggests that the first oncogenic events occur as far back as progenitor cells or HSCs. These observations led us to evaluate the primitive HSC fraction in patients with CLL and we found that the propensity to generate clonal mature B cells was present in HSCs. Although CLL cells were never directly engrafted in xenograft models, HSCs from patients with CLL caused abnormal monoclonal or oligoclonal mature B cells *in vivo*.<sup>111</sup> Moreover, NGS studies confirmed that CD34<sup>+</sup>CD19<sup>-</sup> hematopoietic stem/progenitor cells (HSPCs) and myeloid cells from patients with CLL shared somatic mutations identical to those detected in CLL cells. Such recurrent mutations included *NOTCH1*, *SF3B1*, *BRAF*, *TP53*, *XPO1*, *MED12*, *NFKBIE*, and *EGR2*.<sup>5,112,113</sup> Whole-genome sequencing also confirmed shared mutations between MBL/CLL cells and their respective polymorphonuclear cells, suggesting that the acquisition of some somatic mutations occurs before disease onset, likely at the HSC stage.<sup>114</sup> Moreover, the activation of NOTCH1 pathways is deeply involved in CLL leukemogenesis.<sup>91</sup>



**Fig. 1.** Summary of the pathways and molecules involved in CLL pathogenesis. These deregulated biological pathways are affected by genetic and non-genetic mechanisms, and coordinately drive CLL leukemogenesis.

The NOTCH1 signaling pathway is aberrantly activated in HSPCs from patients with CLL regardless of *NOTCH1* mutation status (compared with the HSPC levels in healthy donors), suggesting that NOTCH1 activation is an early event in CLL leukemogenesis that may lead to the development of aberrant HSPCs in patients with CLL.<sup>113</sup> Consistent with this, advances in the analysis of IgH genes using NGS technology confirmed the presence of independent oligoclonal B cell clones (even in immunophenotypically monoclonal CLL patients).<sup>115</sup> Thus, the initial oncogenic events target self-renewing HSCs in CLL in humans.

Recent studies clarified that the initial oncogenic events target HSPCs in several types of human mature lymphoid malignancies in addition to CLL.<sup>116-119</sup> Several studies using a mouse model of mature lymphoid malignancies revealed that the lymphoma-specific oncogenes expressed in HSPCs can initiate lymphomagenesis more effectively than those expressed in mature B cells,<sup>116,118,120-122</sup> supporting the model of multistep leukemogenesis or lymphomagenesis initiation from HSPCs. These studies provided novel models of leukemogenesis/lymphomagenesis. Thus, the cellular stages of tumor initiation and final transformation are distinct, and the stage-specific oncogenic events coordinately propagate the tumor in humans. Further studies are necessary to elucidate the detailed molecular mechanisms underlying the multistep leukemogenesis/lymphomagenesis of mature lymphoid

malignancies in humans.

### NOVEL DRUGS FOR CLL TREATMENT

Chemoimmunotherapy using anti-CD20 monoclonal antibodies, such as the fludarabine, cyclophosphamide, and rituximab regimen,<sup>123</sup> remains the standard reference treatment for CLL patients aged <65 years with good health and low-risk prognostic factors. Recent advances in the understanding of CLL pathogenesis markedly improved the range of therapeutic applications for CLL treatment. Therapeutic strategies targeting BCR signaling have been developed due to the essential role of BCR signaling in CLL pathogenesis. Furthermore, venetoclax, a BCL2 inhibitor, markedly altered the therapeutic strategy for CLL treatment. The novel drugs, and their target molecules and pathways in CLL are summarized in Figure 2.

Regarding surface molecules, CD19, CD20, and CD52 have been extensively investigated as therapeutic target molecules for CLL. CD19, a member of the Ig superfamily, is a B cell lineage specific surface molecule involved in BCR signal transduction.<sup>124</sup> The expression of CD19 is restricted to the B cell lineage and HSCs and the majority of hematopoietic cells lack its expression; therefore, CD19 represents a specific therapeutic target for B cell malignancies. T cells bearing a chimeric antigen receptor (CAR T cells) have been

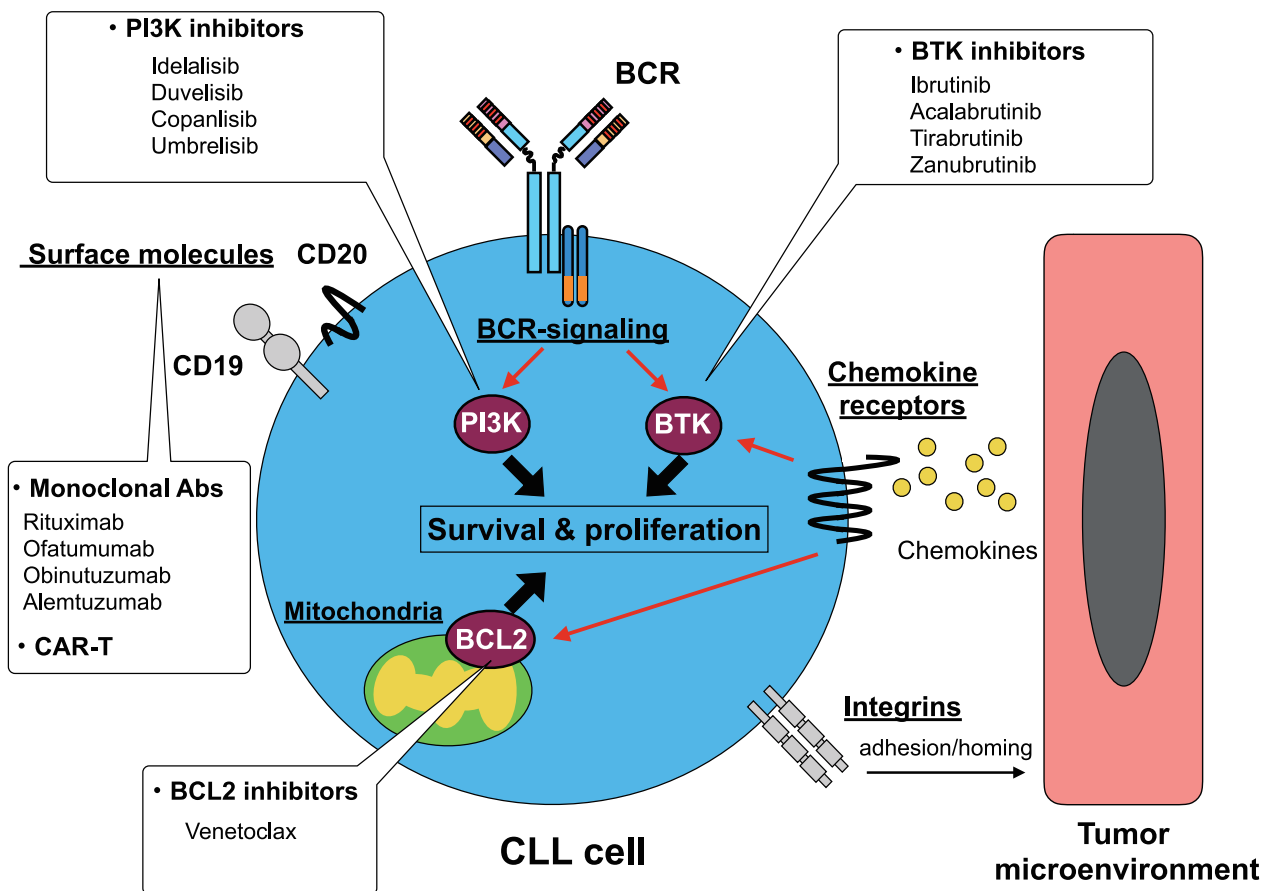


Fig. 2. Summary of novel drugs and their target molecules and pathways.

developed as a new cellular therapy.<sup>125</sup> The most advanced CAR T cells developed to date target human CD19; tisagenlecleucel and axicabtagene ciloleucel. The Food and Drug Administration (FDA) approved tisagenlecleucel for the treatment of refractory B-ALL and refractory diffuse large B cell lymphoma (DLBCL), and axicabtagene ciloleucel for refractory DLBCL. The efficacy of CAR T cells against CLL was first reported in 2011<sup>126</sup> and the number of CLL patients treated using CAR T cells increased in a series of clinical trials.<sup>127-131</sup> CLL was one of the first diseases for which CAR T cells were used; however, the therapeutic experience of CAR T cells is less extensive for CLL due to the relatively lower efficacy against CLL than against B-ALL or DLBCL.<sup>132</sup> CAR T cell therapy is highly effective, but it can induce substantial toxicities such as cytokine release syndrome and neurotoxicity. To overcome these problems, a recent study employed CAR natural killer cells (CAR NK cells), and demonstrated the rapid and persistent efficacy of CAR NK cells against B cell malignancies, including CLL, without the development of major toxicities.<sup>133</sup>

CD20 is a surface glycoprotein expressed on mature B cells and its expression is restricted to the B cell lineage. HSCs and the majority of hematopoietic cells lack its expression; therefore, anti-CD20 monoclonal antibodies, such as rituximab, ofatumumab, and obinutuzumab, have been developed and utilized in the treatment of mature B cell malignancies. Anti-CD20 antibodies are classified into two groups, type I and type II, based on the differences in the epitope and binding mode.<sup>134</sup> Rituximab and ofatumumab belong to the type I group. Type I antibodies can stabilize CD20 molecules on lipid rafts, leading to increased C1q binding and the induction of strong complement-dependent cytotoxicity (CDC). In contrast, type II antibodies, such as obinutuzumab, cannot stabilize CD20 on lipid rafts, resulting in reduced binding potential to C1q and lower levels of CDC; however, they may directly induce cell death.<sup>134,135</sup> A recent study revealed the differential binding mechanisms of therapeutic anti-CD20 antibodies, including rituximab, ofatumumab, and obinutuzumab.<sup>136</sup>

Rituximab, an anti-CD20 chimeric monoclonal antibody, has revolutionized the therapeutic strategies for mature B cell malignancies, including CLL. Rituximab was demonstrated as effective and tolerable as monotherapy for non-Hodgkin's lymphoma (NHL),<sup>137-140</sup> however, rituximab monotherapy was less effective against CLL.<sup>141</sup> In contrast to rituximab monotherapy, chemoimmunotherapy using rituximab, such as the fludarabine, cyclophosphamide, and rituximab regimen, is significantly effective against CLL.<sup>123,142</sup>

Ofatumumab is a human monoclonal anti-CD20 antibody that targets a small-loop, membrane-proximal epitope of the CD20 molecule.<sup>143</sup> Ofatumumab monotherapy is an effective, well-tolerated treatment for fludarabine-refractory CLL patients.<sup>144</sup> The safety and efficacy of combination therapies,<sup>145,146</sup> and maintenance therapy<sup>147</sup> using ofatumumab were investigated in several studies.

Obinutuzumab is a humanized, afucosylated, type II anti-CD20 antibody. A phase 1/2 clinical study revealed that

obinutuzumab monotherapy is effective for patients with heavily pretreated relapse/refractory CLL.<sup>148</sup> A randomized phase 3 trial demonstrated that the addition of obinutuzumab to chlorambucil significantly prolonged overall survival compared with chlorambucil monotherapy in untreated CLL patients not eligible for intensive chemotherapy.<sup>149</sup>

Alemtuzumab is an anti-human CD52 humanized IgG1 monoclonal antibody. A variety of human lymphoid malignancies and normal lymphocytes express CD52 antigens. The efficacy of alemtuzumab was investigated in previously-treated<sup>150,151</sup> and untreated CLL patients,<sup>152,153</sup> and the FDA approved it for the treatment of fludarabine-refractory CLL in 2001. The major toxicities of alemtuzumab treatment include infusion reactions, myelosuppression, and immunosuppression. In particular, infectious events were observed in several clinical trials<sup>151,154</sup> due to immunosuppression by CLL and T cell depletion by alemtuzumab.

In addition to surface molecules, therapeutic strategies targeting BCR signaling have been developed for CLL treatment. Ibrutinib, a Bruton tyrosine kinase (BTK) inhibitor, is an orally bioavailable small molecule that covalently bonds to the cysteine-481 residue of BTK. Ibrutinib exhibited potent activity against previously treated CLL or CLL with *TP53* aberrations,<sup>155-158</sup> and is increasingly used as monotherapy or tested in combination with other regimens. Of note, BTK inhibitors exert anti-CLL activity by inhibiting BCR signaling, and the interaction between CLL cells and their microenvironment.<sup>159</sup> Ibrutinib treatment releases CLL cells from their microenvironment, where they are required for CLL proliferation, into peripheral blood, leading to apoptosis via the downregulation of several adhesion molecules.<sup>160-162</sup> Ibrutinib also alters the immunosuppressive CLL microenvironment by inhibiting signal transducer and activator of transcription 3 pathways.<sup>163</sup> Although it has high clinical efficacy, disease progression during ibrutinib treatment has been reported. Ibrutinib resistance is due to CLL clones harboring mutations in *BTK* and *PLCG2*, a downstream molecule of BTK in the BCR signaling pathway, which drive the clonal expansion of CLL during ibrutinib treatment.<sup>164-167</sup> New BTK inhibitors, such as acalabrutinib, tirabrutinib, and zanubrutinib, have been developed, and their efficacies and safety profiles were clarified in clinical trials.<sup>168-171</sup> Recent studies investigating acalabrutinib, a second generation BTK inhibitor, confirmed the efficacy of combination therapy consisting of acalabrutinib and obinutuzumab in patients with treatment naïve and relapse/refractory CLL.<sup>168,172</sup> Further studies will aid in the development of safe and effective therapeutic strategies using BTK inhibitors.

Phosphatidylinositol 3-kinases (PI3Ks) integrate and transduce signals from BCR, chemokine receptors, and adhesion molecules.<sup>173-175</sup> They are subdivided into classes I, II, and III. Class I PI3Ks comprise four isoforms, PI3K  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ . PI3K $\delta$  expression is primarily restricted to hematopoietic cells, and it plays an essential and nonredundant role in BCR signaling.<sup>176-178</sup> Idelalisib is a potent and selective PI3K $\delta$  inhibitor<sup>179,180</sup> that exerts anti-CLL effects by suppressing BCR signaling, and the interaction between CLL cells

and their microenvironment.<sup>180</sup> Oral idelalisib therapy exhibited a favorable safety profile and rapidly induced stable disease control in most heavily pretreated CLL patients.<sup>181</sup> The combination therapy of idelalisib and rituximab resulted in a higher overall response rate than rituximab monotherapy in relapsed CLL patients.<sup>182,183</sup> Another combination therapy of idelalisib, bendamustine, and rituximab improved progression-free survival compared with bendamustine plus rituximab alone in patients with relapsed or refractory CLL, but an increased risk of infection was reported in the idelalisib group.<sup>184</sup> Next-generation PI3K inhibitors, such as duvelisib, copanlisib, and umbralisib, were previously developed.<sup>185-188</sup> Duvelisib, a dual inhibitor of PI3K $\delta$  and PI3K $\gamma$ , was approved by the FDA for relapsed or refractory CLL/small lymphocytic lymphoma in 2018 based on the results of the phase 3 DUO trial.<sup>189</sup>

In addition to novel drugs targeting BCR signaling pathways, such as BTK and PI3K inhibitors, the BCL2 inhibitor venetoclax has also markedly altered CLL treatment. This BH3 domain mimic prevents the interaction between BCL2 and BH3, and inhibits the anti-apoptotic effects of BCL2. As constitutively activated BCR signaling and the most frequently observed chromosomal abnormality del13q14 cause high levels of BCL2 expression, BCL2 represents a reasonable therapeutic target in CLL. The efficacy and safety of daily oral venetoclax for relapsed or refractory CLL was reported in a phase 1 dose-escalation study.<sup>190</sup> A phase 2 study of venetoclax monotherapy in patients with relapsed or refractory CLL with del17p reported an overall response rate of 79.4% at a median follow-up of 12.1 months.<sup>191</sup> The phase 3 MURANO trial in patients with relapsed or refractory CLL compared the combination of venetoclax/rituximab with that of bendamustine/rituximab therapy, resulting in a 2-year progression-free survival rate of 84.9% and 36.3%, respectively.<sup>192</sup> This study evaluated the minimal/measurable residual disease (MRD) status using multicolor flow cytometry and polymerase chain reaction assays, and the frequencies of the patients who achieved a negative MRD status were significantly higher in the venetoclax/rituximab treatment group than in the bendamustine/rituximab treatment group.<sup>192</sup> Similarly, the recent phase 2 CLARITY trial investigating the combination of ibrutinib and venetoclax for relapsed or refractory CLL reported a high rate of MRD eradication.<sup>193</sup> Based on these studies, an MRD-guided treatment strategy may be the standard of care for CLL in the near future. Further studies will be helpful to establish therapeutic strategies using such novel drugs and improve the clinical outcome of CLL.

## CONCLUSIONS AND PERSPECTIVES

Our understanding of the pathogenesis of CLL has markedly improved in the last decade regarding recurrent mutations, immunological aspects of CLL-BCR, and the initiation of multistep leukemogenesis by HSCs. Furthermore, the development of novel drugs targeting molecules essential for CLL has significantly improved the clinical outcome of CLL

patients. Based on both basic and clinical studies, we plan to further investigate CLL biology to overcome this disease.

## CONFLICT OF INTEREST

The author has no conflicts of interest related to this article.

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