Current Treatment Options in Oncology (2014) 15:187–209
DOI 10.1007/s11864-014-0285-5

Leukemia (JP Dutcher, Section Editor)

Update on the Biology and Treatment Options for Hairy Cell Leukemia

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Published online: 21 March 2014

Keywords Hairy cell leukemia · Pentostatin · Cladribine · Rituximab · Immunotoxins · Vemurafenib · *BRAF* mutations

Opinion Statement

Hairy cell leukemia (HCL) is an uncommon chronic leukemia of mature B cells. Leukemic B cells of HCL exhibit a characteristic morphology and immunophenotype and coexpress multiple clonally related immunoglobulin isotypes. Precise diagnosis and detailed workup is essential, because the clinical profile of HCL can closely mimic that of other chronic B-cell lymphoproliferative disorders that are treated differently. Variants of HCL, such as HCLv and VH4-34 molecular variant, vary in the immunophenotype and specific VH gene usage, and have been more resistant to available treatments. On the contrary, classic HCL is a highly curable disease. Most patients show an excellent long-term response to treatment with single-agent cladribine or pentostatin, with or without the addition of an anti-CD20 monoclonal antibody such as rituximab. However, approximately 30-40 % of patients with HCL relapse after therapy; this can be treated with the same purine analogue that was used for the initial treatment. Advanced molecular techniques have identified distinct molecular aberrations in the Raf/MEK-ERK pathway and *BRAF* (V600E) mutations that drive the proliferation and survival of HCL B cells. Currently, research in the field of HCL is focused on identifying novel therapeutic targets and potential agents that are safe and can universally cure the disease. Ongoing and planned clinical trials are assessing various treatment strategies, such as the combination of purine analogues and various anti-CD20 monoclonal antibodies, recombinant immunotoxins targeting CD22 (e.g., moxetumomab pasudotox), *BRAF* inhibitors, such as vemurafenib, and B-cell receptor signaling inhibitors, such as ibrutinib,

which is a Bruton's tyrosine kinase inhibitor. This article provides an update of our current understanding of the pathophysiology of HCL and the treatment options available for patients with classic HCL. Discussion of variant forms of HCL is beyond the scope of this manuscript.

Introduction

Hairy cell leukemia (HCL) is an uncommon chronic B-cell lymphoid malignancy. HCL is differentiated from other B-cell lymphoproliferative disorders by specific cytomorphological, immunophenotypic, and molecular features. It is characterized by progressive accumulation of clonal mature B lymphocytes with "hairy" projections on the cell surface in the blood, bone marrow, and spleen. In 1958, Bouroncle et al. described 26 cases of leukemic reticuloendotheliosis in which the leukemic cells present in blood and bone marrow exhibited a distinct "lace-like" outline of the cell membrane. These cells were later termed "hairy cells" in 1966 by Schrek et al. [1, 2], who observed undulating ruffles or hairs on the HCL cell surface using a phase contrast microscope. HCL is currently classified by the World Health Organization under B-cell non-Hodgkin lymphoma [3]. Due to its unique pathologic features, HCL has always drawn much attention from medical students, pathologists, and clinicians despite its low frequency of occurrence and excellent response to therapy.

HCL accounts for 2 % of all leukemias. Approximately 1,000 new cases occur every year in the United States. The median age at diagnosis is 58 years, and the male-to-female ratio is 4:1 [4, 5]. HCL is considered a disease of middle-aged adults, although it can occur in very young individuals [6]. HCL is more common among those with European or Ashkenazi Jewish ancestry, and Asian countries have a lower incidence of HCL than Western countries [7]. Elderly and African-American patients have decreased survival durations [8]. A few studies have shown that HCL incidence is higher among individuals with a family history of HCL or chronic lymphocytic leukemia [9, 10]. A common HLA link between family members with HCL has been suggested [11]. A few other conditions also have been reported to be associated with HCL; these include autoimmune disorders, such as vasculitis (specifically polyarteritis nodosa), and exposure to coal dust [12-14]. Exposure to radiation and exposure to cytotoxic agents are not particularly associated with HCL.

Significant strides have been made over the past two decades in the understanding of the pathobiology of HCL and more treatment options are now available for patients with HCL, but for many, the disease remains incurable.

Update on the pathobiology of HCL

Advances in molecular techniques have helped to improve understanding of various aspects of the pathophysiology of HCL.

BRAF mutations

In 2003, two groups demonstrated the role of the mitogen-activated protein kinase (MAPK) pathway in the survival and growth of HCL cells [15•, 16]. In 2011, using whole-exome sequencing, European investigators found mutations in the *BRAF* gene in all 47 patients with HCL who were included in the study [17••]. Recently, *BRAF* mutations were reported to be present in hematopoietic stem cells in patients with HCL [18]. These findings confirmed that the *BRAF* mutation V600E is a molecular hallmark of HCL. Moreover, this mutation was not detected in other B-cell lymphomas or in the variant forms of HCL (HCLv and VH4-34 HCL) [19•, 20, 21].

The *BRAF* gene is composed of 18 exons. The most common *BRAF* mutation occurs at position 1799, in which thymine and adenine are exchanged, resulting in the substitution of glutamic acid with valine at position 600 (V600E) on exon 15 [22]. *BRAF* V600F mutations also occur in other cancers, such as malignant melanoma and papillary thyroid cancer [23]. The *BRAF* gene is a member of the serine/threonine protein kinase family. *BRAF* mutations provide Ras-independent activation of the MAPK pathway, causing hyperactivation of *ERK* and thereby promoting the growth, survival, and differentiation of HCL cells [24]. Various groups have demonstrated *BRAF*

(V600E) mutations using pyrosequencing and other techniques; detection of *BRAF* mutations has been shown to be a useful diagnostic tool for HCL [25, 26].

Anecdotal reports have shown that inhibition of *BRAF* kinase by drugs, such as vemurafenib, is effective in relapsed refractory HCL [27-31]. However, the long-term impact of this strategy is unclear at this time. Reports of resistance to these *BRAF* inhibitors are emerging [32]. Figure 1 shows the Raf-MEK/ERK pathway in HCL, as well as other potential therapeutic targets. Of note, patients with VH4-34 HCL do not exhibit *BRAF* mutations [19•]. Furthermore, it was recently reported that activating mutations of *MAP2K1* occur in HCLv and VH4-34 HCL but not in classic HCL [33].

Cellular origin of HCL B cells

The precise cellular origin of HCL remains elusive. Late-activated post-germinal center memory B cells, and possibly splenic marginal zone B cells, are currently considered the cell of origin for HCL [34, 35]. Gene expression profiling and comparative expressed sequence hybridization studies indicate that HCL cells are more akin to memory B cells and splenic marginal zone B cells [36, 37]. Most HCL B cells (90 %) exhibit immunoglobulin heavy chain (*IGHV*) mutation with >2 % deviation from the germline sequence [38, 39].

B-cell receptor signaling and structure

The role of the antigenic drive (autoantigen or polyreactive antigen) and activation of B-cell receptor (BCR) signaling is currently under extensive investigation in various B-cell lymphoproliferative disorders. Activation of BCR results in stimulation of various intracellular signaling pathways involving kinases, such as *SYK*, *BTK*, and *LYN* kinases, thus stimulating the growth of lymphoid cells (Fig. 1). These signaling pathways are being explored as potential therapeutic targets in HCL [40]. Structurally, one of the unique features of HCL B cells is coexpression of multiple clonally related immunoglobulin isotypes (IgM/IgD, IgG, and IgA), especially IgG3, in 40-80 % cases [41, 42]. This feature provides an ontogenic link between germinal center reaction and development of HCL B cells. HCL B cells preferentially demonstrate the usage of VH3-21, 3–30, and 3–33. Recently, the VH4-34 molecular variant of HCL was described; this variant has an aggressive disease course and an inferior response to treatment with purine analogues [19•].

IGHV mutation status

Similar to chronic lymphocytic leukemia, in HCL, the mutational status of *IGHV* has prognostic implications. Patients with HCL can be divided into two major prognostic subgroups on the basis of the mutational status of *IGHV*: mutated HCL (>2 % variation from germline) and unmutated HCL (≤2 % variation from germline). Patients with unmutated HCL have shorter overall survival durations than those with mutated HCL [39].

Specific chromosomal aberrations

Recurrent chromosomal translocations are rare in HCL. A few studies have assessed the patterns of chromosomal abnormalities in patients with HCL using the array comparative genomic hybridization technique [43, 44]. Chromosomal defects such as 14q32-22, gain of 5q13.31, trisomy 5, and loss of 7q22-q35 were observed in one-third of the patients [45, 46]. Thus, the genomic profile of HCL is generally stable. In contrast, patients with HCLv have del17p.

Other signaling pathways

In addition to activation of the Raf-MEK/ERK pathway, the activation of other signal transduction pathways, such as the PI3K-AKT pathway, could promote HCL cell growth. HCL B cells overexpress IL-3Rα or CD123 and FLT3 (fms-related tyrosine kinase 3) or CD135 on the cell surface; the activation of these pathways can in turn lead to the activation of the PI3K-AKT pathway, which provides an antiapoptotic effect in HCL B cells [36]. Fibroblast growth factor receptor 1 (FGFR1) also is overexpressed in HCL B cells. Basic fibroblast growth factor (bFGF), which is secreted by HCL cells, can provide autocrine growth-promoting and cytoprotective effects in HCL B cells [36, 47]. Protein kinase Cε signaling also contributes to HCL cell survival and the hairy morphology of HCL cells [15•, 48].

Tissue homing, microenvironmental, and cellular influences in HCL

Unlike in other lymphomas, HCL cells rarely accumulate in the lymph nodes and tend to accumulate specifically in the blood, bone marrow, splenic red pulp, and hepatic sinusoids. Various studies have shown that lymphoid tissue microenvironments can propagate the growth of malignant B lymphocytes in various

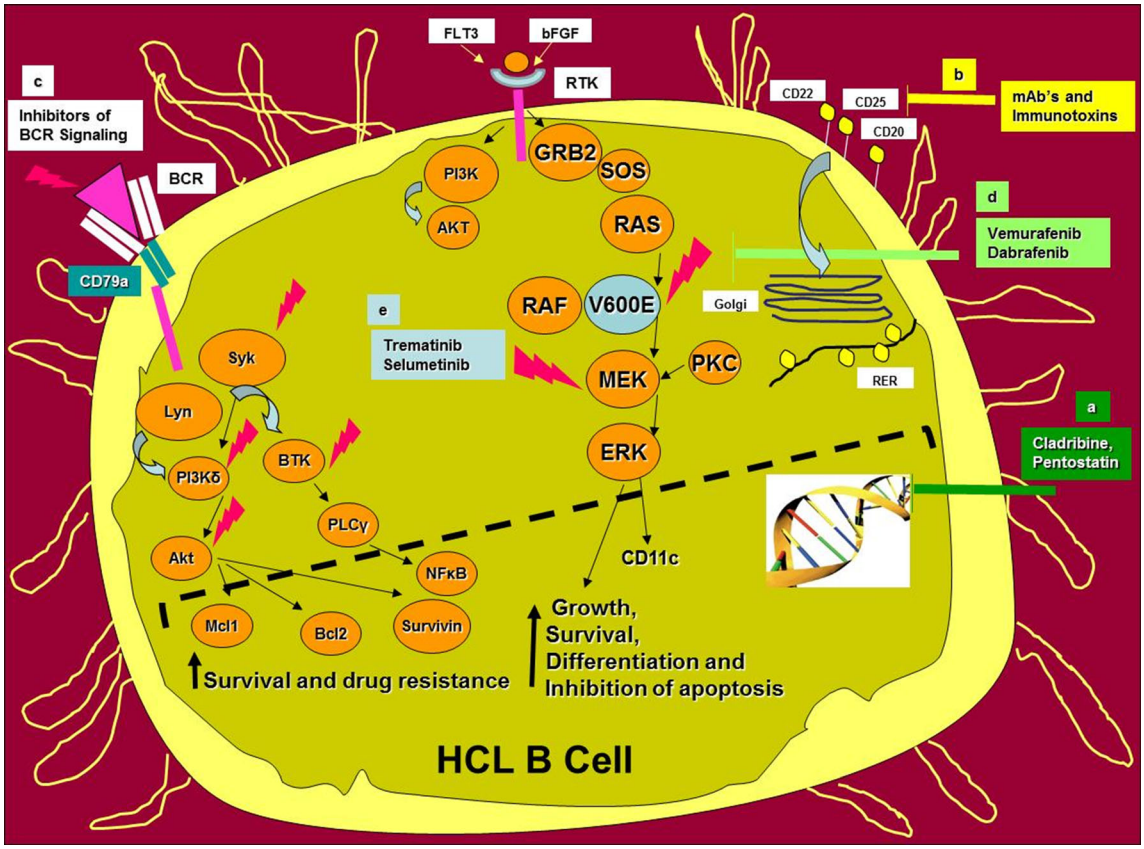

Fig. 1. Mechanism of action of currently used therapies and potential therapeutic targets in hairy cell leukemia (HCL). Intracellular signaling pathways in HCL B cells are shown. B-cell receptor (BCR) and Raf-MEK/ERK signaling are the dominant pathways with major therapeutic implications in HCL. The pink lightning bolt symbol indicates therapeutic targets. (**a**) Purine analogues (shown in dark green). Purine analogues are the most commonly used chemotherapeutic agents in HCL. Cladribine and pentostatin inhibit DNA polymerase and adenosine deaminase, prevent DNA repair, and promote DNA damage. (**b**) Monoclonal antibodies (mAbs) and immunotoxins (shown in yellow). HCL B cells overexpress CD20, CD22, and CD25. These are the common surface epitopes exploited for therapy. Rituximab is an anti-CD20 mAb. Immunotoxins against CD22 and CD25 are in clinical trials. Recombinant immunotoxins combine the binding domain of the plant or the bacterial toxin with the variable region of the mAb. These agents facilitate the entry of an exogenous toxin into the cell cytoplasm, block protein synthesis, and inhibit rough endoplasmic reticulum by ribosylation of the elongation factor. Examples of immunotoxins are BL22 (anti-CD22 mAb fused to pseudomonas exotoxin PE38) and HA22 (moxetumomab pasudotox). (**c**) Inhibitors of BCR signaling (shown in white). BCR is composed of two immunoglobulin heavy and light chains (variable and constant regions) and CD79a, which have an intracellular activation motif that transmits signals to intracellular tyrosine kinases (e.g., Btk, Syk, and Lyn). A Btk inhibitor (ibrutinib) is the most promising treatment for HCL and is currently under evaluation. (**d** and **e**) Inhibitors of Raf-MEK/ERK. Mutations of *BRAF* (V600E) are considered a major factor for HCL cell growth. Raf-MEK/ERK signaling pathways can be blocked by inhibition of *BRAF* (shown in light green) by agents such as vemurafenib or by inhibition of mitogen-activated kinase (*MEK*) by trematinib (shown in blue).

lymphomas [49]. One recent study demonstrated that the cytokine milieu differs between the spleen and lymph nodes, suggesting that these organs may have different functions [50]. The spleen and lymph nodes should be investigated independently in lymphoid malignancies.

Preferential localization of HCL cells in various tissue microenvironments is dependent on overexpression or

underexpression of chemokines, adhesion receptors, and various cytokines (summarized in Table 1). HCL B cells overexpress CXCR4 (CD184) and CD9 and underexpress L-selectin (CD62L), CXCR5 (Burkitt lymphoma receptor 1), and CCR7, which might explain the reduced involvement of lymph nodes in HCL [51•]. In addition, differential expression of various adhesion molecules can explain the specific distribution of HCL cells in bone marrow, splenic red pulp, and hepatic sinusoids. Overexpression of CD49d ($\alpha4\beta1$), CD49e ($\alpha5\beta1$), and VLA-4 (CD49d+CD29) promotes the adhesion of HCL cells to extracellular matrix proteins, such as fibronectin, which is overexpressed on mesenchymal stromal cells and endothelial cells.

Tissue homing of HCL cells to sinusoidal compartments in the spleen and liver could be explained by the interaction between VLA-4 and VCAM-1 (vascular cell adhesion molecule 1 or CD106). HCL-associated splenic lesions are called "splenic psudosinuses," which are formed because of the accumulation of erythrocytes in vascular lesions lined by HCL cells [52, 53]. Similarly, HCL cells also overexpress CD51 ($\alpha_v\beta_3$), which is a receptor for vitronectin, and CD44, which is a receptor for hyaluronan. Binding to vitronectin receptors provides motility for HCL cells and leads to their accumulation in vitronectin-rich splenic red pulp. In contrast, binding of CD44 isoforms (CD44v3 and CD44H) to hyaluronan promotes bone marrow fibrosis (a common occurrence in patients with HCL) by stimulating bFGF-mediated production of fibronectin [54, 55]. In addition, transforming growth factor (TGF)-$\beta1$ produced by HCL cells promotes reticulin fiber formation by stimulating bone marrow fibroblasts, thus contributing to the bone marrow fibrosis [56].

HCL cells also overexpress CD11c, CD103, CD200, CD1d, cyclin D1, and annexin A1, which are essential in diagnosing HCL, although the functional significance of these molecules in the biology of HCL is unclear [57, 58, 36, 59]. Interestingly, HCL cells also overexpress cyclin D1 in the absence of translocation t(11;14), which is typically associated with mantle cell lymphoma [60].

The functional significance of HCL-specific T lymphocytes is unclear but is thought to influence HCL pathobiology. T lymphocytes from patients with HCL have decreased expression of costimulatory CD28 and hence can respond poorly to antigens [61]. These T cells have a very restricted and skewed repertoire (TCR-β) and are not cytotoxic to HCL cells. Another possibility is that these activated T cells can bind to CD40-activated HCL cells and promote disease progression. HCL clones can express synpatojanin-2, which can be recognized by HCL-specific T cells [62, 63].

Levels of various cytokines are elevated in the sera from patients with HCL, influencing the biology of HCL [64]. These cytokines include tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-2Rα (CD25), TGF-β, bFGF, interferon alpha (IFN-α), and granulocyte macrophage colony-stimulating factor. Of note, patients with HCL treated with IFN-α demonstrate rapid clearance of HCL cells from the peripheral blood compared with lymphoid tissues [64, 65]. This differential response can be explained by promotion of TNF-α by IFN-α, which downregulates the antiapoptotic protein IAP (inhibitor of apoptosis) in the peripheral blood; in the lymphoid tissues, the presence of integrins and adhesion molecules prevents the downregulation of IAPs by TNF-α, hence reducing the clearance of HCL cells from tissues [66].

Table 1. Patterns of distribution of chemokines, chemokine receptors, adhesion receptors, and various cytokines and differential localization of hairy cell leukemia (HCL) cells in tissues [51–54]

Involved tissues	Distribution of molecules
Reduced involvement of lymph nodes	Possibly due to downregulation of L-selectin (CD62L), CXCR5, and CCR7
Propensity to involve splenic red pulp and hepatic sinusoids	Overexpression of CD49d and VLA-4
Splenic red pulp—"splenic psudosinuses"	Overexpression of vitronectin receptor (CD51)
Bone marrow fibrosis	Overexpression of CD44 isoforms, TGF-β, and bFGF
Slow clearance of HCL cells from tissues in response to treatment with IFN-α	Integrins prevent the downregulation of IAP molecules by TNF-α, which is induced in response to treatment with IFN-α, thus preventing apoptosis of HCL cells

Cross-talk among T cells, HCL B cells, the CXCR4-CXCL12 axis, and various cytokines can support HCL cell growth, proliferation, and survival. Understanding the function of various immune cells and signaling molecules in HCL is an active area of current research [67].

What are hairy cells?

The term "hairy cell" is derived from the presence of undulating, irregular projections, and clumps of short microvilli on the surface of HCL cells. The ultrastructure of these cells was further studied using electron microscopy [68]. The abundance of cytoplasm rich in mitochondria, along with ribosomal-lamellar complexes, suggests that HCL cells are metabolically very active. Factors responsible for the morphology of HCL cells are membrane projections rich in F actin (polymerized actin), constitutive activation of Rho-GTPases, and overexpression of proteins, such as CD9, pp52, and GAS7 (growth arrest–specific 7), which crosslinks the F-actin and aggregates it in the filamentous projections [69, 36, 70]. The presence of various adhesion receptors also helps in the motility, homing, and adherence of HCL cells in different tissue compartments.

Clinical features of HCL

In general, classic HCL has an indolent disease course. Patients commonly present with asymptomatic cytopenias (anemia, neutropenia, monocytopenia, or thrombocytopenia). Fatigue, recurrent infections, and bleeding, which are symptoms related to cytopenias, abdominal distension due to organomegaly (splenomegaly and hepatomegaly), and spontaneous splenic rupture also can occur. Constitutional symptoms, such as fatigue, fever, night sweats, and weight loss, may or may not be present. Increased incidence of infections (herpes viruses, bacterial pathogens, and *Pneumocystis jiroveci*) may occur due to immune function defects. Rarely, some patients can present with bulky lymphadenopathy, lytic bone lesions, osteosclerosis, cutaneous lesions, leukocytoclastic vasculitis, autoimmune hemolytic anemia, central nervous system involvement, soft tissue infiltration, ascites, or pleural effusion [13, 71-74]. Occasionally, patients with HCL can develop other lymphoid and nonlymphoid malignancies [75, 76].

Diagnosis

Investigations

Complete blood count, differential, and platelet counts and careful review of a peripheral blood smear are the most appropriate initial investigations. Most patients (70-90 %) exhibit pancytopenia: low white blood cell count (<5,000/μL), low hemoglobin levels (<10 g/dL), neutropenia (<1,000/μL), monocytopenia (<100/μL), and low platelet count (<100,000/μL). Rarely (10-20 % of patients), leukocytosis (white blood cell count >10,000/μL) may be observed. Abnormal liver and renal function may be observed in some patients. Serum lactate dehydrogenase levels are usually normal. Serum soluble IL-2R (sCD25) levels are elevated in patients with HCL, and these levels are correlated with disease activity. Detailed flow cytometry analysis of peripheral blood and/or bone marrow aspirate to detect the specific immunophenotype of HCL cells is essential for the diagnosis and exclusion of other lymphoproliferative disorders. Bone marrow trephine biopsy is required. Other tests that should be performed at the time of diagnosis include tests for serum immunoglobulin levels, somatic *IGHV* mutations, VH gene usage, and *BRAF* mutations, specifically V600E.

Factors predicting inferior outcomes include the presence of abdominal lymphadenopathy, anemia or thrombocytopenia, unmutated *IGHV*, VH4-34 gene usage, *TP53* gene mutations, partial response to treatment with purine analogues, and early relapse (<1-2 years) after therapy [39,

71, 77•, 78, 79•]. The persistence of minimal residual disease (MRD) after treatment with purine analogues may or may not predict relapse [80].

Histopathologic and immunophenotypic features of HCL

Peripheral blood/bone marrow flow cytometry of lymphocytes

Lymphocytes from a patient with classic HCL show strong expression of CD19, CD20, FMC-7, CD11c, CD103, CD25, HC2, CD22, sIg, CD79a, and CD123 and are generally negative or dim for CD5, CD23, CD10, CD79b, and CD27. Clonality is suggested by kappa or lambda light chain restriction on HCL cells [81]. Using four main surface markers (CD11c, CD103, CD25, and CD123/HC2), Matutes et al. [82] proposed a scoring system for the diagnosis of HCL: 1 point is given for each positive marker and 0 points for each negative marker. A score of 3–4 was obtained in more than 98 % of patients with HCL, and a score of 0–2 was obtained in patients with HCLv and splenic lymphoma with villous lymphocytes. Recently, expression of CD200 and CD1d was shown to be consistent in HCL, which is useful for differential diagnosis [58, 57]. Table 2 provides a brief summary of the main differentials of classic HCL [81].

Bone marrow biopsy –

Bone marrow biopsies in patients with HCL frequently result in dry tap, because HCL is usually associated with bone marrow fibrosis. This appears to be due to overexpression of FGFR1 and TGF-β by HCL cells [47, 83]. Bone marrow biopsies from patients with HCL can exhibit a "fried egg" appearance due to wide spaces between the nuclei and abundant and clear cytoplasm spread out diffusely in HCL cells [56, 84]. Immunohistochemical analysis is performed for CD20, TRAP (tartrate-resistant acid phosphatase), DBA-44, and annexin-A1. Of note, in one study, the annexin-A1 gene was specifically upregulated in HCL [36]. HCL cells also can be positive for cyclin D1 and TIA-1 (T cell intracytoplasmic antigen-1). T-bet transcription factor staining is helpful to detect minimal HCL involvement in the bone marrow [60, 85, 86]. Reticulin staining is generally moderate to strongly positive and identifies bone marrow fibrosis.

Other tissues

HCL predominantly involves splenic red pulp areas with atrophy of white pulp. Dilated vascular spaces in the spleen of a patient with HCL are called splenic pseudosinuses [87]. Occasionally, hepatic sinusoids and lymph nodes can be involved in HCL.

Detection of MRD

Assessment for MRD is important in patients with HCL in clinical trials [88]. It is unclear whether MRD positivity can clearly predict inferior long-term outcomes or early relapses in patients with HCL. Two reports have suggested that the presence of MRD can predict relapse [89, 90]. In another study of 19 patients who were in complete hematologic remission and had a median follow-up period of 16 years, 9 patients (47 %)

Table 2. Differential diagnosis of classic hairy cell leukemia (HCL) [81]

Characteristic	HCL	HCLv	Splenic marginal zone lymphoma/ splenic lymphoma with villous lymphocytes	B-cell prolymphocytic leukemia	HCL (Japanese variant)	CLL	Splenic diffuse red pulp lymphoma
Morphology	Medium size, uneven villi, indistinct nucleoli	Medium size, uneven villi, distinct nucleoli	Small size, polar villi, small nucleoli	Medium size, no villi, prominent nucleoli	Medium size, uneven villi, indistinct nucleoli	Small size, smudge cells, no villi	Small size, villi present, small nucleoli
White blood cell count	Low	High	High	High	High	High	Low
Monocytopenia	Yes	No	No	No	No	No	-
Lymphadenopathy	No	Yes	Yes	Yes	Yes	Yes	Yes
Marker profile							
CD25	++	Neg	Neg	Neg	Neg	Neg	Neg
CD103	++	++	Neg	Neg	-	Neg	Neg
CD11c	++	++	+	+	++	+	+
CD123	++	Neg	Neg	Neg	-	+	Neg
CD5	Neg	Neg	Neg	+	Neg	++	Neg
CD23	Neg	Neg	Neg	+	Neg	++	Neg
Annexin-A1	++	Neg	Neg	Neg	-	Neg	Neg
Sox-11	+	-	Neg	-	-	Neg	-
Cyclin D1	+	-	Neg	-	-	Neg	Neg
HCL score	3-4	0-2	0-2	0-2	0-2	0-2	0-2
Histologic findings							
Spleen	Red pulp	Red pulp	Marginal	White and red pulp	Red pulp	White and red pulp	Red pulp
Bone marrow	Interstitial	Interstitial	Nodular to intrasinusoidal	Diffuse	Interstitial	Diffuse	Interstitial to diffuse, absent pseudosinus

became MRD-negative after treatment with single-agent cladribine. However, even some of the patients who had morphological evidence of HCL had prolonged survival [80]. Flow cytometry for key HCL markers, immunohistochemical analysis for HCL markers, and assessment of *BRAF* (V600E) mutations using next-generation sequencing or allele-specific polymerase chain reaction are techniques that could be used for detection of MRD in HCL.

Clinical management

At the time of initial diagnosis, patients with HCL can be asymptomatic or can present with cytopenias, recurrent infections, fatigue, and painful splenomegaly. For asymptomatic patients, a wait and watch approach is feasible with proper counseling regarding infections and symptoms of disease progression. Symptomatic patients should be treated.

Update on treatment options for HCL

Currently available treatment options are summarized in Table 3. Treatment with a single-agent purine analogue (cladribine or pentostatin) with or without rituximab is the most common strategy used by clinicians [77•, 91••, 92]. Some of the treatment challenges include MRD, relapsed disease, second cancers, and refractory disease.

Table 3. Reported outcomes for commonly used treatment options for hairy cell leukemia (primary or relapsed)

Regimen	No.	Objective response rate, %	Complete remission rate, %	Comment
Single-agent cladribine (0.1 mg/kg per day for 7 days, continuous infusion) [78, 101]	349	98	91	96 % of patients were alive after 4 years; relapse rate was 16 % for those who achieved complete remission and 54 % for those who achieved a partial response; outcomes were better in patients who achieved complete remission
Single-agent cladribine (0.1 mg/kg per day for 7 days, continuous infusion) [104]	85	100	79	87 % of patients were alive after 12 years; 36 % experienced relapse after 10 years of follow-up
Single-agent cladribine (0.12 mg/kg per day, 2-hour intravenous bolus for 5 days vs. every week for 6 weeks) [107]	68 64	95 91	76 72	Similar response and survival outcomes with both schedules of cladribine
Single-agent pentostatin (4 mg/m^2 intravenously every 2 weeks for 6 months) [94•]	154	79	76	This randomized trial confirmed the superiority of pentostatin over IFN-α; after 9 years of follow-up, 83 % of patients were alive[99]
Single-agent pentostatin (4 mg/m^2 intravenous bolus once every 2 weeks until maximum response or failure) [131]	238	96	79	Estimated 5-year and 10-year overall survival rates of 89 %
Single-agent cladribine (5.6 mg/m^2 intravenously over 2 hours daily for 5 days followed by 375 mg/m^2 intravenous rituximab weekly for 8 weeks) [91••]	31	100	100	After a median follow-up period of 2 years, median progression-free and overall survival durations had not been reached

Treatment with purine analogues

Patients with HCL respond very well to treatment with single-agent pentostatin or cladribine [78, 93, 94•, 95••, 96]. Few studies have examined the activity of fludarabine [97]. No randomized trials have compared pentostatin with cladribine; however, a retrospective study from the Royal Marsden Hospital compared the outcomes of patients treated with pentostatin (n=188, median follow-up period of 14 years) with those of patients treated with cladribine (n=45, median follow-up period of 9 years) [77•]. This study demonstrated equivalent overall response rates (96 % for pentostatin, 100 % for cladribine), complete remission (CR) rates (82 % for pentostatin, 76 % for cladribine), and 10-year overall survival rates (100 % for pentostatin, 86 % for cladribine). Relapse rates were similar for both groups (44 % for pentostatin, 38 % for cladribine). Furthermore, patients who achieved CR and recovered their hemoglobin and platelets had significantly longer relapse-free survival durations than those who achieved a partial response (PR) and had incomplete recovery of hemoglobin and platelets. In multivariate analysis, response to treatment was the most significant predictor of relapse-free survival. At 16-year follow-up, 91 % of the patients who were alive were in remission and 79 % had achieved CR [77•].

Pentostatin (2'deoxycoformycin)

In 1984, Spiers et al. [96] reported that two patients with HCL had achieved CR after treatment with pentostatin [98]. Several other studies have confirmed the efficacy of pentostatin in achieving prolonged CR in patients with HCL. Various dose schedules of pentostatin have been investigated, with similar results. The United States Food and Drug Administration approved pentostatin for the treatment of HCL (4 mg/m^2 intravenously once every 2 weeks until maximum response). Pentostatin is safe in patients with creatinine clearance >60 ml/minute, but dose reduction is needed if creatinine clearance is between 40 and 60 ml/minute.

One randomized study demonstrated the superiority of pentostatin over IFN-α in terms of CR and objective response rate (76 % vs. 11 %; 79 % vs. 38 %, respectively). Relapse-free survival was significantly longer in patients treated with pentostatin [94•]. In a follow-up report, the estimated 10-year relapse-free survival rate in patients treated with pentostatin who achieved CR was 67 %; pentostatin also was effective in patients whose treatment with IFN-α failed [99]. Ten-year overall survival rates ranged from 80 % to 90 % in different studies of pentostatin. Common toxicities associated with pentostatin were grade 3–4 neutropenia and thrombocytopenia (10-20 %). Several reports also demonstrated that treatment with pentostatin is associated with a reduction in absolute CD4+ and CD8+ T cells, which recover several months after completion of therapy without any long-term sequelae [100]. The combination of pentostatin with rituximab is promising. A retrospective study in patients with relapsed HCL (n=12) showed that pentostatin combined with rituximab led to a 92 % CR rate and a 100 % overall response rate and improved disease-free survival durations compared with pentostatin alone [92].

Single-agent cladribine

Cladribine has demonstrated considerable activity in HCL as a single agent (CR rate of 80-98 % in most studies). Cladribine causes lymphocytotoxicity in HCL cells through the accumulation of DNA strand breaks, activation of DNA polymerase, and promotion of apoptosis [98]. Investigators from Scripps Clinic initially demonstrated the efficacy of single-agent cladribine in HCL [95••]. A single cycle of cladribine was administered (0.1 mg/kg per day by continuous intravenous infusion for 7 days) in 12 patients with previously treated HCL. Eleven of the 12 patients (92 %) achieved CR in the first 2 months of treatment. Later, the same group reported excellent results in 349 patients with HCL (179 were previously untreated) [78, 101]. Ninety-one percent of patients achieved CR and 6 % achieved a PR. The responses were independent of prior therapy. Relapse rates were lower in patients who achieved CR (16 %) than in those who achieved a PR (54 %). Twenty-six percent of patients experienced relapse after a median follow-up period of 29 months. The overall survival rates were 98 % at 48 months and 97 % at 9 years (108 months). A second course of cladribine was administered in 53 patients at the time of first relapse and 33 (62 %) achieved CR.

Recently, the Scripps Clinic group reported responses and outcomes in 88 patients aged 40 years or younger at diagnosis who were treated with single-agent cladribine [102]. This study reported that these young patients responded equally well and had similar outcomes to those of older patients (historical cohort). Forty-five of 83 evaluable patients (54 %) relapsed after first-line cladribine compared with 37 % in the historical cohort. No statistically significant increase in second cancers appeared in the young patients, as was observed in the overall cohort. The median overall survival duration was 21 years. Two other reports have demonstrated 12-year overall survival rates of 79 % (n=44) and 87 % (n=86) after treatment with cladribine [103, 104].

The main toxic effects associated with cladribine include neutropenia (65-85 % of patients) and thrombocytopenia (20 % of patients). Febrile neutropenia was observed in 40 % of patients. In a phase II study of 35 patients with HCL, filgrastim was administered with cladribine (3 days before cladribine and 2 days after completion of therapy) [105]. Administration of filgrastim did not improve the incidence and duration of febrile events, suggesting that cladribine may induce fever by release of cytokines from HCL cells. A variety of cladribine dosing strategies have been investigated, including subcutaneous and intravenous administration routes, daily or weekly dosing, and different durations of infusion; all have shown a similar efficacy and safety profile [106, 107].

Approximately 30-40 % of patients experience relapse after treatment with either cladribine or pentostatin, and 60-70 % of these patients can achieve a second CR or PR depending on the duration of the first CR. If relapse occurs within the first 2 years of treatment with purine analogues, then the chance of response to retreatment with the same agent decreases [77•, 108]. Because single-agent rituximab can produce CR in 10-54 % of patients with relapsed HCL [109, 110], strategies involving the combination of rituximab with purine analogues were explored.

Chemoimmunotherapy: combination of rituximab with cladribine or pentostatin

The rationale for adding rituximab to purine analogues was based on the efficacy and safety of this combination in patients with relapsed disease, as well as the significant synergy of this combination observed in other lymphoproliferative disorders [97, 111].

We have reported a phase II clinical trial in 31 previously untreated patients with HCL [91••]. Cladribine was administered at 5.6 mg/m^2 intravenously over 2 hours per day for 5 consecutive days; approximately 4 weeks after initiating cladribine, 8 weekly doses of rituximab (375 mg/m^2 intravenously) were administered. A CR rate of 100 % was reported, and after a median follow-up period of 25 months, the median CR duration, progression-free survival duration, and overall survival duration had not been reached. Patients achieved MRD-negative status after completion of treatment with rituximab, as demonstrated by flow cytometry and consensus polymerase chain reaction. No severe grade 3–4 events occurred (except for reversible grade 3–4 infections, which occurred in 12 patients). Despite a significant decline in the number of CD4+ and CD8+ T cells, as well as serum immunoglobulin levels, no increase in the incidence of opportunistic infections was observed. A longer follow-up of this study may provide more information about the importance of achieving MRD-negative CR in long-term outcomes.

Another group reported the results of a retrospective study of 18 patients with relapsed HCL [92]. Patients received pentostatin (n=12) or cladribine (n=6) in combination with rituximab. A CR rate of 89 % was observed, and after a median follow-up period of 3 years, 16 patients maintained CR. The estimated recurrence rate was 7 %, compared with the 55 % recurrence rate noted after first-line treatment with single-agent pentostatin or cladribine. Similarly, a combination of oral fludarabine and rituximab was tested in 15 patients with relapsed HCL [97]. All patients responded; the 5-year progression-free survival rate was 89 % and the 5-year overall survival rate was 83 %.

Krietman et al. also have studied bendamustine in combination with rituximab in 12 patients with relapsed HCL [112]. The dose of bendamustine was 70 or 90 mg/m^2 on day 1 and day 2 of each cycle, and 375 mg/m^2 rituximab was administered on day 1 and day 15 of the cycle. CR was achieved in approximately 60 % of patients at a median follow-up period of 31 months [113]. Results of combination studies are promising, and longer follow-up with larger numbers of patients will determine whether this strategy will replace the current practice of using single-agent cladribine or pentostatin as the frontline therapy for HCL.

Treatment response assessment

Response to therapy is assessed 3–5 months after completion of therapy. CR is defined by the absence of circulating hairy cells in the peripheral blood and bone marrow and resolution of hepatosplenomegaly and cytopenia. Immunohistochemical analysis of the bone marrow should be negative for CD20- or DBA-44–positive cells. PR is defined by resolution

of cytopenia, ≥50 % decrease in organomegaly (if previously enlarged), and ≥50 % decrease in bone marrow infiltration, without any hairy cells in the blood.

Novel therapeutic agents

As our understanding of the pathobiology of HCL has improved, new therapeutic targets have been identified (summarized in Table 4). Agents targeting these molecular pathways are under investigation and some have demonstrated significant activity in patients with relapsed disease. Figure 1 depicts various agents and their mechanism of action in the HCL cell.

Inhibitors of BRAF and the MAPK pathway

The *BRAF* (V600E) mutation is the driver mutation for HCL cells and has been reported to be universally present in classic HCL. Specific inhibitors targeting the *BRAF* pathway have been evaluated. In vitro studies have demonstrated that HCL cells undergo significant apoptosis after incubation with *BRAF* inhibitors [114]. Clinical trials of *BRAF* inhibitors to treat HCL are motivated by results from the use of *BRAF* inhibitors to treat metastatic melanoma.

Vemurafenib

Vemurafenib has demonstrated significant activity in patients with metastatic melanoma in which the *BRAF* (V600E) mutation plays a major role in melanoma cell growth. Vemurafenib is an oral agent that inhibits the thymidine kinase enzyme and specifically targets cells containing *BRAF* (V600E) mutations [115]. Anecdotal case reports have demonstrated the activity of vemurafenib in patients with relapsed HCL [28-30, 116]. In the four reported cases, varying doses of vemurafenib were used (240–960 mg twice per day) and patients achieved a morphologic and molecular CR 1–3 months after starting therapy. Recently, the preliminary results of a phase II trial of vemurafenib in five patients with relapsed HCL were reported [31]. Vemurafenib (960 mg) was administered twice per day continuously for 4 weeks for 3 cycles. Two patients achieved marrow CR and three achieved marrow PR, and all patients achieved complete hematologic recovery. The main toxic effects were skin rash, photosensitivity, and arthralgia. One patient developed a squamous cell carcinoma of the skin, which was completely resected. Four patients needed a dose reduction to 480 mg twice per day due to toxicity. Plasma levels of inflammatory cytokines and *BRAF*+HCL cells decreased dramatically, and levels were correlated with hematologic responses.

Table 4. Promising therapeutic agents for HCL [114, 116, 118, 120, 125]

Agent	Mechanism of action
Vemurafenib, dabrafenib	Inhibition of *BRAF*
Ibrutinib	Bruton's tyrosine kinase inhibitor
Trematinib, selumetinib	MAPK (mitogen activated protein kinase) inhibitors
Moxetumomab pasudotox	High-affinity anti-CD22 immunotoxin

One of the concerns with the use of vemurafenib is development of second cancers, cutaneous lesions, and relapses due to activation of the MEK pathway. Therefore, other agents, such as dabrafenib, are now being studied [117]. Another strategy is to combine *BRAF* inhibitors with *MEK* inhibitors (such as trematinib) [118]. It was shown that in patients with melanoma, progression-free survival durations were better after treatment with a combination of dabrafenib and trematinib than with dabrafenib alone. Patients with HCLv and VH4-34 variants of HCL have mutations in the MAPK pathway [33]; therefore, this combination strategy might be useful for patients with HCLv or VH4-34 variant HCL, which generally do not respond well to purine analogues.

Immunotoxins

Monoclonal antibodies (mAb) against various targets have been developed for the treatment of various B-cell lymphoid malignancies. To circumvent issues of monoclonal antibody resistance and to cause maximum cell death, researchers are seeking other mechanisms. Developments in bioengineering techniques, phage typing, and cloning now allow us to develop antibody-drug or antibody-toxin conjugates.

One such approach is to develop immunotoxins. This term refers to a fusion of a truncated plant or a bacterial toxin (pseudomonas or diphtheria exotoxin) to the variable region of a monoclonal antibody that is directed against a specific cell surface target, such as CD25 or CD22 in the case of HCL [119]. The binding domain of the immunotoxin is missing, and instead, the carboxy region of the variable chain of the mAb is fused with the toxin, resulting in an immunotoxin. This hybrid agent contains mAb to identify and bind to a specific cell target and the truncated toxin, which is released inside the cell to block protein synthesis. Once the toxin is released inside the cell, it is internalized to the endoplasmic reticulum by the KDEL receptor (endoplasmic reticulum protein retention receptor-1) and the catalytic or enzymatic domain causes ADP ribosylation of elongation factor 2 (EF-2) and finally blocks protein synthesis (Fig. 2) [120].

A few clinical trials from the National Cancer Institute have studied the efficacy of recombinant immunotoxins against CD22 (BL-22 and moxetumomab pasudotox) and CD25 (LMB-2) in patients with refractory HCL [121, 122•, 123]. In a phase II clinical trial, immunotoxin BL22 was tested in 36 patients with relapsed refractory HCL [122•]. After one cycle (40 μg/kg every other day, 3 doses), the CR rate was 25 % and the objective response rate (ORR) was 50 %, and these improved to a 47 % CR rate and a 72 % ORR after retreatment (only in patients with cytopenias). Patients who had undergone splenectomy or had previously experienced massive splenomegaly had an inferior CR rate (21 %) and ORR (36 %) compared with those without massive splenomegaly (64 % CR rate and 95 % ORR). Two patients developed reversible grade 3 hemolytic uremic syndrome that did not require plasmapheresis.

To improve the efficacy and safety of BL22, the binding affinity of the immunotoxin to CD22 was improved by identifying a mutant (with 3

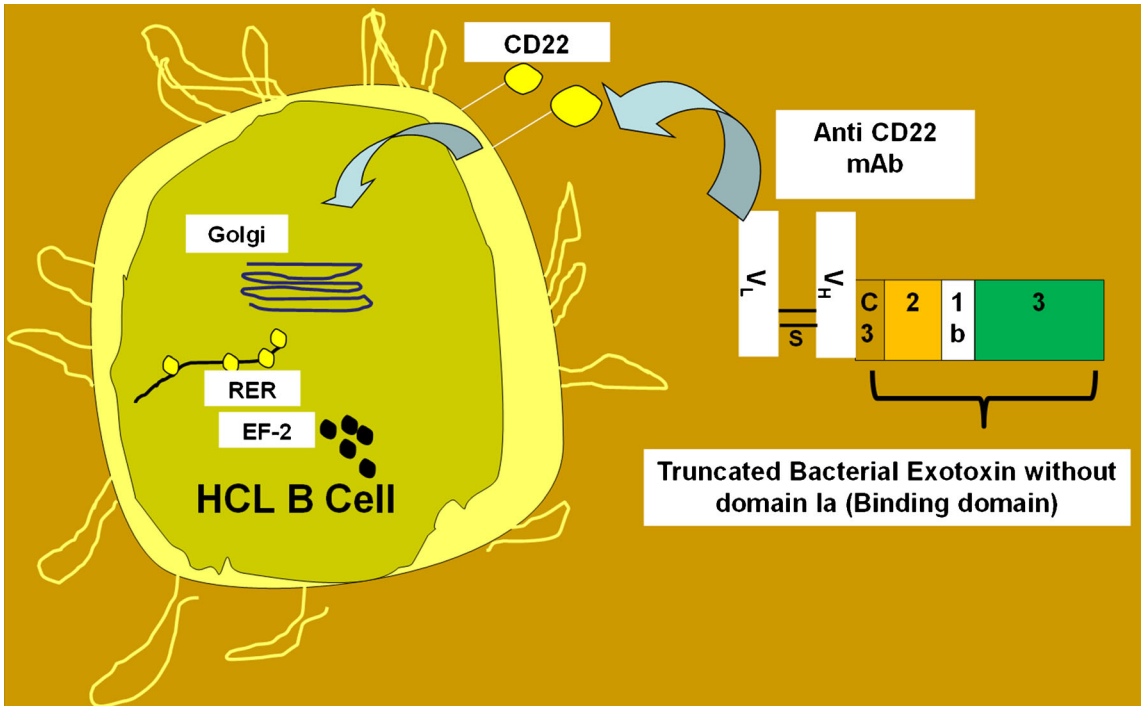

Fig. 2. Structure and mechanism of action of immunotoxins in hairy cell leukemia (HCL). The binding domain of the bacterial exotoxin is replaced with the variable region of the heavy chain (V_H) of the monoclonal antibody (in this case against CD22). After the internalization of the immunotoxin, the enzymatic domain (domain 3) ribosylates elongation factor 2 (EF-2) and inhibits protein synthesis. The affinity of the immunotoxins can be increased by mutations at CDR3 (third complementarity determining region), allowing higher binding of the immunotoxin to HCL cells.

different amino acids) in the complementarity determining region (CDR3) of the variable heavy chain of the monoclonal antibody. This immunotoxin is known as moxetumomab pasudotox, or HA22 [121]. An update of the phase I dose escalation trial of moxetumomab pasudotox in 49 patients with relapsed refractory HCL [124] showed a CR rate of 57 % and an ORR of 88 %. Among patients who received a high dose (50 μg/kg every other day, 3 doses; n=33), 64 % achieved CR and 13 of 21 achieved MRD-negative CR, maintained after a median of 32 months. Two patients developed grade 2 hemolytic uremic syndrome, which was reversible without specific treatment. Patients who had previously undergone splenectomy achieved partial remission. Patients with the lowest HCL burden appeared to have a better chance for durable CR.

BTK inhibitor ibrutinib

Ibrutinib has shown excellent results in chronic lymphocytic leukemia and mantle cell lymphoma [125]. A clinical trial with single-agent ibrutinib in patients with relapsed refractory HCL is ongoing (NCT01981512).

Flavopiridol –

In an anecdotal report, flavopiridol, which is a pan–cyclin-dependent kinase inhibitor, led to CR in a patient with refractory HCL. Treatment

with flavopiridol was complicated by tumor lysis syndrome, which was managed successfully [126].

Other treatment options

These were some of the earliest treatment options utilized for HCL, preceding the use of purine analogues.

Splenectomy

This modality is used rarely. A few studies have reported the beneficial effects of splenectomy. Currently, splenectomy can be indicated for patients with a painful and massively enlarged spleen (>10 cm below the left costal margin) and minimal bone marrow infiltration by HCL cells, or for patients in whom all other modalities have failed. The responses to splenectomy do not last for more than 5 years, but some patients can achieve very long-term remission [127, 128].

Interferon alpha (IFN-α)

Currently, the role of IFN-α in the treatment of HCL is limited. Previous studies have demonstrated that IFN-α can improve response rates, with most patients achieving PR. HCL cells are very sensitive to treatment with IFN-α, but studies have shown that the duration of remission was shorter and relapses occurred when IFN-α was discontinued [94•]. Toxic effects associated with IFN-α included flu-like symptoms, fatigue, central nervous system effects, depression, second cancers, and neuropathy. Approximately 20 % of patients may develop a second cancer [129]. Pentostatin was found to be superior to IFN-α in terms of responses, safety, and outcomes [94•].

Stem cell transplantation

Only a few anecdotal case reports have shown that allogeneic stem cell transplantation can be very helpful in patients with advanced-stage, multiple refractory HCL [130].

Conclusions

Most patients with classic HCL have done extremely well after treatment with single-agent purine analogues. However, approximately 40 % of patients still experience relapse and can develop refractory disease. Increased understanding of the molecular defects promoting HCL cell growth, such as *BRAF*/*MAPK* and BCR signaling, helped to identify novel therapeutic targets. With improvement in molecular techniques, variants of classic HCL such as VH4-34 variant were identified. Ongoing clinical trials of *BRAF* inhibitors immunotoxins, ibrutinib, and new anti-CD20 monoclonal antibodies have shown promising preliminary results in patients with refractory HCL. How these new strategies will impact the long-term, relapse-free survival and overall survival of patients remains to be seen. Furthermore, longer follow-up of clinical studies is needed to understand the significance of MRD-negative CR in patients with HCL.

Therefore, enrollment in clinical trials should be encouraged and patients should be counseled regarding the rationale of these strategies. Continuous research is needed to further understand the biology of HCL and recognize various pathways that can be manipulated therapeutically.

Compliance with Ethics Guidelines

Conflict of Interest
Preetesh Jain, Naveen Pemmaraju, and Farhad Ravandi declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

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