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Hairy Cell Leukemia

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Introduction

The WHO categorizes hairy cell leukemia (HCL) as a mature B-cell neoplasm. HCL is characterized by lymphocytes with prominent cytoplasmic projections (hairy cells) infiltrating the bone marrow and spleen, leading to pancytopenia, bone marrow fibrosis, and splenic enlargement. Hairy cells have a unique immunophenotypic profile—CD11c+, CD20+, CD25+, and CD103+—that confirms its diagnosis. The course of HCL is usually chronic, but can often be progressive, and most patients require treatment at some point. The purine nucleoside analogues, pentostatin and cladribine, are highly active, but cladribine is the preferred first-line choice due to its efficacy, brief treatment duration, and favorable toxicity profile. Other therapeutic options include rituximab, interferon-alpha, vemurafenib, and splenectomy. With current therapy, an overall survival of 87% at 12 years has been reported.

History

HCL was originally recognized in the 1920s but was not identified as a unique entity with distinct pathological and clinical characteristics until 1958 when Bouroncle and colleagues characterized it as *leukemic reticuloendotheliosis* [1] and described the first 26 cases. In their landmark article, the authors provided a comprehensive description of the clinical course, pathology, and limited treatment at the time with alkylating agents and splenectomy. The term "hairy cell leukemia" was first coined by Schreck and Donnelly in 1966 when they noted hairlike cytoplasmic projections on phase-contrast microscopy [2]. The last 50 years, and especially the last two decades, have been spent defining HCL as a B-cell neoplasm [3, 4] and have heralded dramatic therapeutic advances with the purine nucleoside analogues.

Epidemiology and Etiology

HCL is uncommon and accounts for 2–3% of all adult leukemias in the USA [5]. According to the Surveillance Epidemiology and End Results (SEER) database, 2856 cases were diagnosed between 1978 and 2004 [6]. There is a 4:1 male predominance and the median age at presentation is 50 years [5]. New data suggest a bimodal incidence pattern, with an early peak around age 40 years and a later peak at 80 years [6]. The disease is more common in Caucasians, with an increased incidence in Ashkenazi Jewish men.

No well-defined etiology for HCL has been reported. Case reports have suggested an association with farming, woodworking, and exposure to organic solvents [7]. A recent hospital-based case-control study in France noted significant associations between HCL and organochlorine insecticides, and phenoxyacetic and triazine herbicides, though the numbers in the study were small [8]. Infectious etiologies such as EBV and HTLV-1 have also been postulated as causes [9, 10]. Familial cases of HCL have been rarely reported. Makower et al. described two cases of familial HCL. In one case, a 50-year-old man developed HCL and a year later his mother was diagnosed with the same entity. In the other family, an aunt of a patient with HCL was diagnosed with Hodgkin's disease. Interestingly, in both families, the younger generation developed the hematologic malignancy at an earlier age. This phenomenon, known as anticipation, has been noted in other malignancies [11]. Cases of familial HCL have also identified HLA haplotypes specific to each family. Each family's HLA haplotype was unique and there has been no identification of a common HLA haplotype among unrelated cases of HCL [11, 12].

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Pathogenesis

Ontogeny

With the advances in molecular techniques, the ontogeny of HCL is becoming clearer. The hairy cell phenotype is that of a late B-cell precursor, likely an activated memory B cell, with aberrant gene expression [13]. The post-germinal center origin is supported by the presence of Bcl-6 mutations and somatic point mutations in the immunoglobulin variable region of the heavy chain [14, 15]. Furthermore, hairy cells express several pan B-cell markers including CD19, CD20, and CD37, but are devoid of the early markers of B-cell development, including CD21 and CD 24 [16]. Hairy cells express the plasma cell antigen-1 (PCA-1) but lack expression of PC-1 which appears later in B-cell ontogeny. This observation suggests that hairy cells do not differentiate into terminal B cells, i.e., plasma cells [3]. DNA microarray analysis illustrates a homogeneous phenotype distinct from other B-cell malignancies. When compared to normal B cells, hairy cells share many genes with memory B cells involved in proliferation and apoptosis [4].

Adhesion/Homing

Hairy cells are highly adherent and can spontaneously bind to several matrices, including fibronectin, vitronectin, and hyaluronan [17, 18]. This binding is facilitated by specific adhesive proteins on hairy cells, including the integrins α4β 1, α5β1, and αvβ3 [18]. Hairy cells characteristically disseminate into the red pulp of the spleen and hepatic sinusoids and portal tracts, but spare lymph nodes [19]. Not only do hairy cells infiltrate many different types of tissues, but they also modify the tissues they infiltrate. Thus, they cause bone marrow fibrosis and form vascular lakes (pseudosinuses) in the spleen [20]. This modification is inherent to the tissue matrix and is enhanced by hairy cell interactions [18]. For example, fibronectin is important in the development of bone marrow fibrosis and it is thought that hairy cells themselves are intricately involved in its production and assembly [17]. Recently, gene analysis has provided more insights into hairy cell adhesion and targeting. For instance, the lack of hairy cell lymph node infiltration can be explained by downregulation of CCR7, a chemokine receptor that allows B cells to enter lymph nodes. Also, hairy cells remain confined to blood-related compartments due to upregulation of genes that prevent their extravasation [4].

Cytogenetics

No karyotypic abnormality is pathognomonic for HCL. Clonal karyotypic abnormalities are variable and range from 20 to 67% of patients [21]. Unlike most other B-cell malignancies, HCL lack balanced chromosomal translocations which occur with immunoglobulin gene rearrangements that are switched off in memory B cells [13]. Instead, chromosomal gains, deletions, and inversions have been identified. In one study, 40% of karyotypic abnormalities involved chromosome 5, with aberrations in band 5q13 being most common [21]. Other chromosomal abnormalities include deletion of 14q and losses of the long arm of chromosome 7 [22, 23]. Evaluation by FISH has revealed that p53 deletions, a marker found in aggressive disease, occur in HCL. The clinical significance of this finding in an indolent disease is currently under investigation [24].

Diagnosis

Histopathologic and morphologic evaluation of the bone marrow is key to establishing the diagnosis of HCL [25]. Classical cytochemical stainings such as tartrate-resistant acid phosphatase (TRAP) have generally been supplanted by modern diagnostic techniques of flow cytometry and immunohistochemical (IHC) staining.

Cytology

Hairy cells are uniform and monotonous in their appearance [25]. A typical hairy cell is slightly larger than a mature lymphocyte with a distinct nucleus that is usually ovoid, but can also be slightly indented [25]. Unlike other B-cell malignancies, the chromatin is uniformly granular without clumping [26]. Morphologically, hairy cells display features suggestive of a metabolically active cell [27]. They have variable amounts of blue-gray cytoplasm and abundant mitochondria and ribosomes. Hairy cells exhibit thin cytoplasmic "hair-like" projections often appearing as serrated borders (Fig. 10.1). Phase-contrast microscopic studies of live cells show that the surface of these cells is in a constant state of change, reflecting ongoing cytoskeletal and signaling activity [13, 28].

Rarely, ribosomal lamellar complexes, or broad-shaped inclusions, can be seen in the cytoplasm on electron microscopy. These organelles are thought to originate from the endoplasmic reticulum and are characterized by alternating layers of ribosome-like granules and fibrous lamellae [29, 30]. Present in half of the cases, the ultrastructural inclusions are not unique to HCL and have been noted in other lymphoid malignancies [30]. They are of unclear clinical significance [31].

Hairy cell cytoplasm stains strongly for TRAP [32]. Isoenzyme 5 acid phosphatase present in hairy cell cytoplasm resists decoloration with tartrate [33]. Most other lymphoid cells, monocytes, and myeloid cells stain variably for

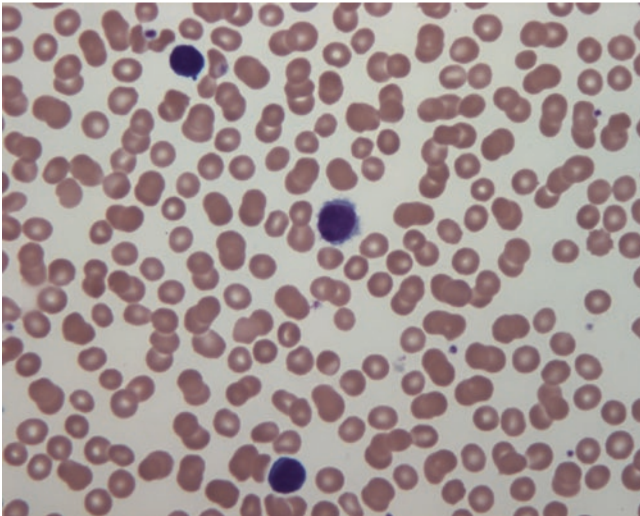

Fig. 10.1 Peripheral blood smear from a patient with HCL. The hairy cell is slightly larger than a mature lymphocyte with ovoid nuclei. Hairy cells characteristically have abundant, *gray-blue* cytoplasm with thin “hairlike” projections (×1000) (corresponds to figure pb 1000×)

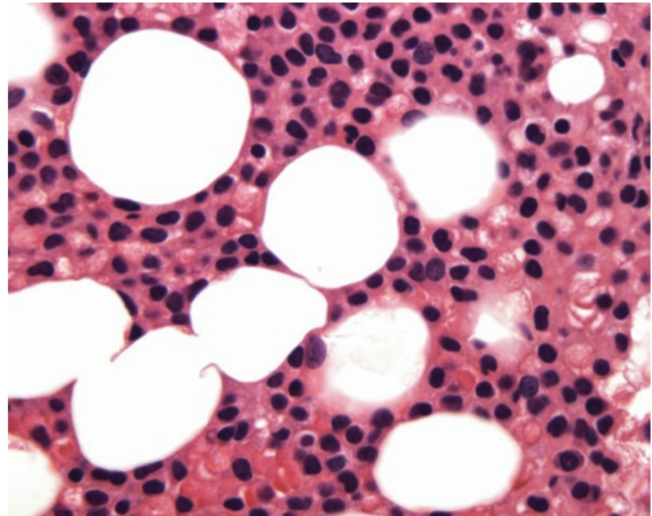

Fig. 10.2 Hairy cell leukemia in the bone marrow, characterized by well-spaced lymphocytes with a “fried-egg” appearance due to the distinct round-to-oval nuclei, which are centrally placed within a pale-staining cytoplasmic domain (×1000) (corresponds to figure bm 1000×)

acid phosphatase activity in the absence of tartrate [16]. TRAP staining is labor intensive and difficult to perform in paraffin-embedded tissues and it is rarely used in the era of immunophenotyping.

Histopathology

Blood and Bone Marrow

Abnormalities in the hemogram are classically seen at presentation in HCL patients [26]. Pancytopenia is common and reported in 80% of patients. Leukopenia is frequently noted [5]. Circulating monocytes are usually absent from the peripheral blood. Despite findings of marrow fibrosis, leukoerythroblastosis is not seen. Circulating hairy cells are variable and oftentimes very difficult to identify [26].

Bone marrow involvement is seen in nearly all patients with HCL [34]. It is often difficult or impossible to obtain an aspirate [25]. The biopsy can show a hypercellular picture. Hairy cells demonstrate patchy or diffuse infiltration of the marrow. A closer examination of the infiltrate reveals a distinctive wide-spaced separation of cells with a surrounding halo, often referred to as a “fried-egg” appearance (Fig. 10.2) [34]. This loose packing of cells results from hairy cells adhering to the reticulin–fibronectin network. Few fibroblasts are seen and trichrome staining does not show deposition of mature collagen [26]. The residual hematopoietic tissues exhibit nonspecific changes [34]. Other collection of cells including small lymphocytes, plasma cells, and mast cells is often identified. Not uncommonly, HCL produces a

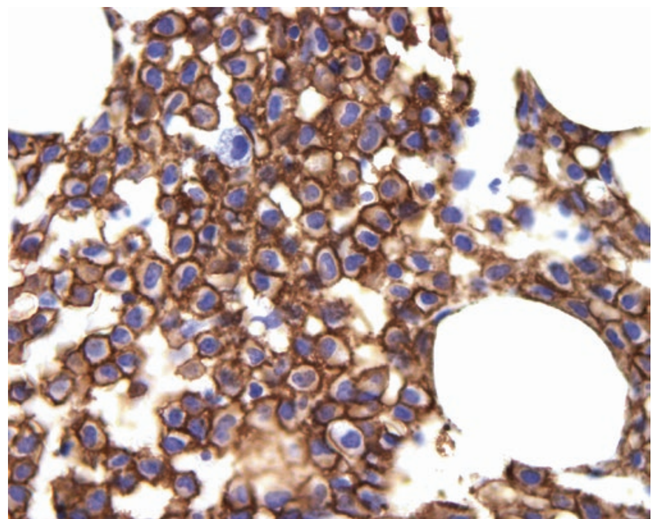

Fig. 10.3 Immunoperoxidase staining with anti-CD20 (B-cell marker), demonstrating strong membrane positivity (×1000). This stain is very useful in evaluating MRD in bone marrow specimens (corresponds to figure bm cd20 1000×)

hypocellular marrow which can be difficult to distinguish from aplastic anemia. Immunostains with CD20 may be helpful (see Fig. 10.3) [35].

Spleen and Liver

Splenic sequestration of hematopoietic elements is a characteristic feature of HCL [36]. HCL mostly affects the red pulp. On microscopy, there is a heavy infiltration of monotonous

cells in the expanded red pulp, sometimes making the individual cords and sinuses indistinguishable. The white pulp atrophies overtime [36]. Hairy cells replace endothelial cells that line the splenic sinusoids and merge to form congested splenic lakes, often appearing as hemangiomas [20]. Remodeling is thought to occur when hairy cells directly network with endothelial cells via integrin receptors and the vitronectin matrix of the basement membrane [13]. Such splenic findings are striking and can sometimes be seen in the bone marrow.

Similarly in the liver, hairy cells infiltrate the hepatic sinuses and portal tracts but spare the parenchyma. They also form characteristic lesions but appear more as angiomas than pseudosinuses since they lack circumferential ring fibers [20].

Genetic Features

With the use of whole-exome sequencing, Tacci et al. recently detected the BRAF V600E mutation in an entire cohort of 48 HCL patients. The absence of this variant in 195 patients with other peripheral B-cell lymphomas or leukemias established it as a key genetic lesion in HCL [37]. The oncogenicity of this mutation results from constitutive activation of the RAF-MEK-ERK mitogen-activated protein kinase pathway. Subsequent studies have confirmed the presence of the BRAF V600E mutation in HCL, with two exceptions [38, 39]; the molecular variants HCL-variant [38] and HCL with IGHV4–34 immunoglobulin rearrangement [39] lack this mutation. Thus, while distinct in the indolent lymphoproliferative disorders, the BRAF V600E mutation has yet to be incorporated into the diagnostic criteria for HCL.

Downstream of BRAF is MEK1, a dual-specificity kinase encoded by MAP 2 K1 (mitogen-activated protein kinase kinase 1). Mutations of the MAP 2 K1 gene have recently been identified in classical, variant, and IGHV-34-expressing HCL patients and appear to be mutually exclusive of the BRAF V600E mutation [40]. Aside from isolated reports [41, 42], MAP 2 K1 mutations in other hematologic malignancies have not been reported. This seemingly unique mutation makes it an attractive target for therapeutic manipulation and warrants further investigation.

Immunophenotyping: Flow Cytometry

Hairy cells can be identified by multicolor flow cytometry to a high degree of certainty even when they compose less than 1% of circulating lymphocytes [43, 44]. They display a mature B-cell phenotype and express pan B-cell markers including CD19, CD20, CD22, and CD 79A [16]. One or more heavy chains and a single light chain are displayed on the cell surface [45, 46]. Frequently, hairy cells demonstrate the presence of surface IgG, specifically the IgG3 isotype, and do not undergo normal B-cell differentiation with class switching [47]. Three markers of importance in the characterization of HCL include CD11c (common in myelomonocytic cells), CD25 (the IL-2 receptor), and CD103 (the alpha subunit of the alpha-beta integrin in intraepithelial T cells) [48–50]. Though these markers are not limited to HCL and can be seen in other lymphoproliferative disorders, such as splenic marginal zone lymphoma (SMZL), their co-expression is unique. For instance, CD11c is distinguished from other disorders by its nearly 30-fold higher intensity of expression in HCL [47, 49]. Moreover, CD103 has the greatest sensitivity and specificity for HCL [26, 51]. Researchers have evaluated the predictive value of the composite phenotype of these antigens. A scoring system was developed by the Royal Marsden Group using the markers: CD11c, CD25, CD103, and HC2 (HCL-associated antigen involved in cell differentiation). Ninety-eight percent of the evaluated cases of HCL had a score of 3 or 4 [52]. Also of note, primarily due to its potential therapeutic implications, is CD52, a marker that has recently been identified in both variant and classical HCL [53].

Further distinctions between variant and classical HCL can be made via flow cytometry. CD123, which is the alpha chain of the IL-3 receptor, is positive in the majority of classical HCL and dim or negative in variant HCL [54–56]. Additionally, CD25 has been shown to be commonly absent in HCL variant [56].

Immunophenotyping: Immunostains

Monoclonal antibodies with specificity for HCL are useful diagnostic tools. They can be performed easily in peripheral blood and paraffin-embedded tissues, and are thus valuable in the evaluation of minimal residual disease (MRD) in treated patients [16]. In addition to the routine B-cell markers like CD20 and PAX5, specific markers for HCL include TRAP, DBA.44, and cyclin D1 [27, 57, 58]. DBA.44 recognizes an unknown fixation-resistant B-cell antigen that is expressed in mantle zone lymphocytes, reactive immunoblasts, and monocytoid B cells [57]. It reacts strongly with HCL (Fig. 10.4) [59]. Although DBA.44 is expressed in other low-grade B-cell lymphoproliferative disorders, a recent study suggests that the combination of DBA.44/TRAP staining has a 97% specificity for HCL [60]. Moreover, CD20 immunostaining is a useful marker in quantifying disease, as it often highlights HCL infiltrates not detected on routine hematoxylin and eosin staining [61].

Annexin A1 (ANXA1) has been identified as a gene that is upregulated in HCL. One study evaluated samples of 500 B-cell tumors for the anti-ANXA-1 monoclonal antibody and found the assay to be both highly sensitive and specific

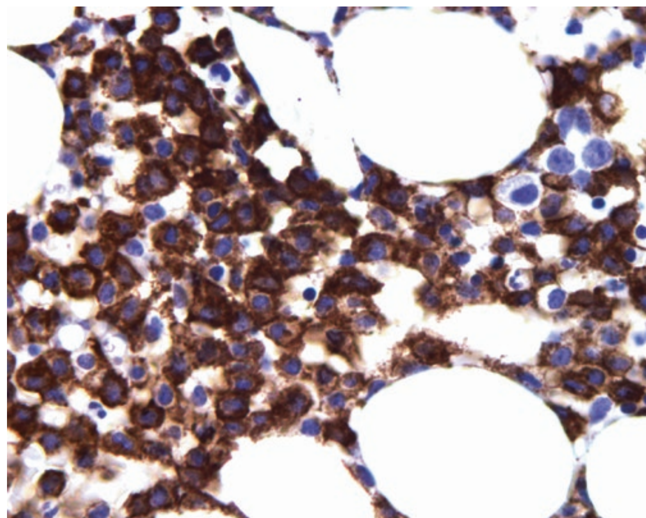

Fig. 10.4 Immunoperoxidase staining with DBA.44. in bone marrow of HCL patient. DBA.44 reacts strongly with HCL. A combination of DBA.44/TRAP staining has a 97% specificity for HCL (corresponds to figure dba-44 1000×)

for HCL (100%) [62]. This precision was not reproduced in a subsequent study, in which only 74% of HCL cases stained positive for ANXA1. Interestingly, none of the HCL variant or BRAF V600E-mutated HCL cases stained for ANXA1 [56].

Clinical Features

General

The onset of HCL may be insidious and its course chronic. It is characterized by pancytopenia and in particular monocytopenia, splenomegaly, and impaired immunity without significant lymphadenopathy [36]. This unique clinical presentation reflects the leukemic infiltration of hairy cells in the bone marrow, spleen, and liver.

In the original description of HCL, fatigue and weakness were the most common symptoms on initial presentation [1]. Also, frequently noted are symptoms of an opportunistic infection and abdominal fullness from splenomegaly. Some patients are incidentally found on physical examination or laboratory workup [5].

On physical examination, splenomegaly is the most prominent finding seen in 80–90% of patients. Spleen size may be variable, but sometimes can be massive [34]. Older studies have suggested that massive splenomegaly, along with patient age and hemoglobin concentration, is associated with a worse prognosis [63]. When present, hepatomegaly usually accompanies splenomegaly, and is seen in 50% of patients [5]. Palpable peripheral lymphadenopathy, unlike other chronic lymphoproliferative disorders, is not common [64]. Internal adenopathy is recognized in one-third of patients with HCL and is thought to be related to disease duration and may correlate with overall survival [64, 65].

Infectious Complications

Infections are a common complication in HCL and a cause of death throughout its course [36, 45]. Among multiple case series, the incidence of serious infections has ranged from 20 to 47%, which includes pneumonia and septicemia [36, 66, 67]. Pyogenic organisms consist of *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterococcus* species [68]. A higher frequency of intracellular organisms such as *Legionella pneumophila* and *Mycobacterium kansasii* has also been noted and thought to arise from defects in monocytes and decreased dendritic cells [45, 69]. Multiple studies have chronicled neutropenia and monocytopenia as contributing causes of the immunodeficiency in HCL [5]. A study of 73 long-term patients found that baseline lymphopenia may be a prognostic factor of increased risk of infectious complications [67].

Secondary Malignancies

Patients with HCL are at increased risk of secondary malignancies [70–72]. Secondary cancers have been attributed to decreased T-cell function from treatment as well as immunologic aberrations from the underlying disease itself [70, 73, 74]. In their 20-year experience with HCL, Wing et al. noted that 22% of their 117 patients developed second malignancies. Cancer risk peaked at 2 years after the diagnosis of HCL and then steadily declined [73]. The authors in this study conclude that HCL patients may be prone to secondary malignancies from the HCL tumor burden rather than genetic predisposition or the immunosuppressive effects of treatment.

Long-term data suggest that secondary cancers are only moderately increased with exposure to purine nucleoside analogues. In their extended follow-up of HCL patients treated with cladribine at Scripps Clinic, Goodman et al. noted 58 second malignancies in 379 treated patients [75]. A subsequent study at the same institution evaluated 83 patients ≤40 years with HCL treated with cladribine; though the excess frequency of developing a second primary malignancy was 1.60 (95% confidence interval, 0.80–2.89), it was not statistically significant [76]. In a retrospective analysis of 487 patients with HCL treated with purine nucleoside analogs, Cornet et al. reported an increased incidence of second malignancies, especially hematological malignancies (standardized incidence ratio 1.86, CI 1.34–2.51 for all malignancies; 5.32, CI 2.90–8.92 for hematological malignancies) [74]. The National Cancer

Institute (NCI) quantified second cancer incidence and cause-specific mortality among 3104 survivors of HCL between 1973 and 2002. They found that the rate of second cancers was 32% compared to the expected 23% in the general population [70].

Other

Extremely rare manifestations of HCL include cutaneous, bone, serosal, and meningeal involvement [77]. Hypocholesterolemia and elevated liver function tests are disease-related findings in HCL [78, 79]. Polyclonal and monoclonal gammopathies have also been noted in 3–20% of patients and can be associated with plasma cell disorders, lymphoma, or autoimmune processes. Autoimmune-associated disorders include polyarthritis nodosa and leukocytoclastic vasculitis [78, 80].

Differential Diagnosis

HCL must be distinguished from other chronic lymphoproliferative disorders that present with splenomegaly and cytopenias, such as hairy cell leukemia variant (HCL variant), splenic lymphoma with villous lymphocytes, and prolymphocytic leukemia. This distinction is critical since these different disorders have unique management approaches and respond quite differently to treatment with interferon-alpha and purine nucleoside analogues.

The differential diagnosis is based on morphologic and phenotypic criteria (Table 10.1).

Hairy Cell Leukemia Variant

HCL variant is a very rare B-cell lymphoproliferative disorder with features distinct from HCL. Patients with HCL variant

Table 10.1 HCL differential diagnosis

Lymphoid malignancy	Clinical characteristics (age/sex)	Morphology	Peripheral blood count	Immunophenotype	Genetics	Spleen	Survival
HCL	Median age: 50 years	Cytoplasm: Irregular	Neutropenia and lymphopenia	CD11c:+++	BRAF V600E mutation ++/–	Red pulp	87% at 12 years
	Male predominance: 4:1	Nucleus: Reniform	Monocytopenia:+	CD25: ++			
		Nucleolus: Not present		CD 103:+++			
				TRAP: ++			
				CD 123 ++			
				ANXA1 +/–			
HCL-variant	Median age: 80 years	Cytoplasm: Irregular	Lymphocytosis	CD11c:++	BRAF V600E mutation ––/––	Red pulp	Median: 9 years
	Male predominance: <2:1	Nucleus: Round	Monocytopenia:–	CD25: ––/–			
		Nucleolus: Present		CD 103:++			
				TRAP: +/–			
				CD 123 +/––			
				ANXA1 –/–			
SMZL	Median age: 65 year	Cytoplasm: Irregular	Lymphocytosis	CD11c:+		White pulp	80% at 5 years
	No gender predominance	Nucleus: Round	Monocytopenia:–	CD25: +/–			
		Nucleolus: Often present		CD 103:+/–			
				TRAP: +/–			
				CD123 +/–			
				ANXA1 –/–			
CLL	Median age: 70 years	Cytoplasm: Smooth	Lymphocytosis	CD11c:+/–			Median: 3 years
	Male predominance: <2:1	Nucleus: Round	Monocytopenia:–	CD25: –			
		Nucleolus: Present		CD 103: –			
				TRAP: –			

+ present, – absent
HCL hairy cell leukemia, *SMZL* splenic marginal zone lymphoma, *CLL* chronic lymphocytic leukemia

are often diagnosed in their seventh or eighth decades and unlike HCL lack a strong male predominance. They typically present with splenomegaly and high leukocyte counts. Though cytopenias may be noted, neither monocytopenia nor neutropenia is a feature of HCL variant [81, 82]. The bone marrow and splenic histologies are similar to those of HCL [81]. Morphologically, cells of HCL variant are intermediate between HCL and B-cell prolymphocytic leukemia [83]. The cells lack monotony and are more varied in appearance. The nucleus is well circumscribed with a prominent nucleolus similar to that of prolymphocytes, while the cytoplasm is more basophilic [81]. Akin to the morphology, the diagnostic profile for HCL variant can be quite distinct. TRAP staining is variable and often negative. Considering the three markers that are characteristically expressed in HCL, CD11c is strongly positive, CD103 is positive in 60% of cases, and CD25 is negative [83, 84].

Examination of the immunoglobulin heavy-chain (IGH) rearrangements and somatic hypermutation patterns has noted significant differences between HCL and HCL variant. In fact, the mutation status of HCL variant mirrored splenic marginal zone lymphoma (SMZL) more than HCL. Specifically, IGHV4–34 was overrepresented in patients with HCL variant and SMZL [85]. Exploration of IGHV4–34 overexpression has established it as a distinct and separate entity from HVL variant, predicting an even worse prognosis [86, 87].

With an aggressive clinical course and refractoriness to traditional therapy, the median survival of patients with HCL variant is 9 years [84]. In addition, approximately 5–10% of patients have transformation to a large-cell process, characterized by significant leukocytosis, B symptoms, and an overall poor prognosis [88]. Moreover, a Japanese variant of HCL has also been described with large granular lymphocytosis [89].

Splenic Marginal Zone Lymphoma

SMZL is a chronic B-cell lymphoproliferative disorder characterized by splenomegaly and lymphocytosis with more polar villous projections. These cells are smaller than hairy cells and have more condensed chromatin [90]. When the hairy cell scoring system is applied to SMZL, the score is usually low [52]. CD103 is rarely positive in SMZL, and in most studies, CD25 and CD11c were positive in 25–47% of cases [91]. The immunologic profile of SMZL is very similar to that of HCL variant and can present a diagnostic challenge. Histologically, SMZL can be distinguished from HCL and HCL variant by splenic expansion of the white pulp and appearance of nodularity in the bone marrow [26].

SMZL usually has a more indolent clinical course with reported 5-year overall survivals of 80% [90].

B-Cell Prolymphocytic Leukemia

B-cell prolymphocytic leukemia is frequently noted in elderly males with prominent splenomegaly without significant lymphadenopathy [92]. The presenting WBC is elevated and often greater than 100×10^9/L with predominant prolymphocytes [93]. Like SMZL, B-cell prolymphocytic leukemia shares many similarities with HCL variant. B-prolymphocytes are larger lymphocytes with a condensed chromatin and a prominent central nucleolus. Immunophenotypically, they are CD25, CD103, and CD11c negative. Overall, they have a poor prognosis and their median survival is 3 years [94].

Treatment

General

Though HCL is an indolent disease, most patients ultimately require treatment [66]. Generally, patients are treated for worsening cytopenias (hemoglobin <10 g/dL, platelet count $<100 \times 10^9$/L, or absolute neutrophil count $<1.0 \times 10^9$/L), infectious complications, and symptomatic splenomegaly. Other less common reasons include bulky lymphadenopathy, progressive visceral or bony disease, or significant autoimmune processes.

Purine Nucleoside Analogues

For many years, splenectomy and interferon-alpha were the standard therapeutic approaches to HCL. The purine nucleoside analogues, cladribine [2-chlorodeoxyadenosine (2-CdA)] and pentostatin (2′-deoxycoformycin), came into clinical use in the mid-1980s and are considered to be the cornerstone of HCL therapy. The discovery that adenosine deaminase (ADA) deficiency produced lymphopenia in children with combined immunodeficiency syndrome led to the development of purine nucleoside analogues [95]. ADA is the major pathway for deoxypurine nucleoside degradation. Resistance to or inhibition of ADA can lead to a buildup of intracellular purine nucleotides which are very toxic to lymphocytes [96–98]. Cladribine (substrate analogue) and pentostatin (direct inhibitor) were developed to oppose the action of ADA.

Cladribine (2-CdA)

Cladribine is commonly chosen as the initial therapy because of its brief treatment administration and high, durable response rates. As a purine nucleoside analogue, it is resistant

to ADA. Cladribine accumulates in lymphoid cells because they have high levels of deoxycytidine kinase [98]. This enzyme phosphorylates cladribine, creating a deoxynucleotide which is then incorporated into DNA, thereby inducing DNA strand breaks and inhibiting repair. Cladribine's potency in indolent lymphomas is a function of its cytotoxicity to both dividing and nondividing lymphocytes [99].

Cladribine was first reported to be effective for HCL in 1990. Under the leadership of Ernest Beutler, investigators at Scripps Clinic first reported on 12 HCL patients treated with a single 7-day course of cladribine at 0.1 mg/kg/day by continuous intravenous infusion. Of those 12 patients, 11 achieved a complete response and the responses were maintained for 16 months [100].

The largest single-institution series was at Scripps Clinic and evaluated 349 previously treated and untreated HCL patients [71]. After a median follow-up of 52 months, 91% of patients achieved a complete response and 7% a partial response. Ninety patients (26%) had relapsed at a median of 29 months. The median survival rate at 48 months was 96%. Rosenberg et al. subsequently reported on 83 patients aged 40 years or less [76]. After a median follow-up of 251 months, 88% of patients achieved a complete response and 12% a partial response. Forty-five (54%) of patients who achieved a response ultimately relapsed at a median of 54 months. Median overall survival for all patients following the first cladribine course was 231 months. The authors hypothesized that the variation in survival data may be attributable to the intrinsic biologic differences between young and old HCL patients.

In the 25-year interval since the introduction of cladribine, many studies have acquired long-term patient data (Table 10.2). Among the assessable patients with HCL treated with cladribine as a single 7-day continuous infusion, complete responses have ranged between 76 and 100%. The majority of these patients have enjoyed long-term remissions with relapse rates of 14% at 24 months and 36% at 9.7 years [101, 102]. In one of the longest follow-up studies, 85% of the patients were alive at 20.9 years [76].

Standard cladribine dosing is a 7-day continuous infusion at a dose of 0.1 mg/kg/day. Alternative treatment schemes have been developed in the hopes of ameliorating prolonged myelosuppression and obviating the need for a pump. Alternative schedules have included a 5-day 2-h infusion at a dose of 0.14 mg/kg/day, weekly 2-h intravenous infusion, subcutaneous administration, and oral administration. Several studies have shown that the 2-h 5-day infusion is equally efficacious with a similar toxicity profile [111]. Robak et al. conducted a prospective study of 132 patients, comparing cladribine administered in a weekly versus daily schedule. Patients were randomized to receive either cladribine 0.12 mg/kg as a 2-h intravenous infusion daily for 5 days or 0.12 mg/kg in a 2-h intravenous infusion once a week for 6 weeks. Results of the trial showed similar complete remission rates, progression-free survival, and overall survival between the two groups. Despite prior reports showing improvement in infectious complications with the weekly dosing, there was no significant difference in grade 3 or 4 infections [112]. Similar results were noted in a Swiss study that compared subcutaneous daily 2-CdA with weekly treatment [113]. Though treatments with these alternative schedules appear promising, they lack the support of long-term follow-up, and the 7-day continuous infusion and 2-h 5-day infusion of cladribine are both considered standard.

Neutropenic fever is the principal acute toxicity of cladribine therapy in HCL, occurring in 42% of treated patients [71]. Infectious complications include bacterial and opportunistic infections. Immunosuppressive effects of

Table 10.2 Long-term follow-up studies with cladribine

Study	Patients (no.)	Median F/U (years)	Initial complete remission rate (%)	Relapse rate (%)	Median time to relapse (months)	Overall survival
Seymour et al. [103]	46	2.5	78	20	16	NA
Hoffman et al. [104]	49	4.6	76	20	NA	95% at 4.6 years
Goodman et al. [75]	209	7	95	37	42	97% at 9 years
Jehn et al. [105]	44	8.5	98	39	48	79% at 12 years
Chadha et al. [106]	86	9.7	79	36	35	87% at 12 years
Else et al. [101]	45	16	76	38	NA	100% at 15 years
Rosenberg et al. [76]	83	20.9	88	54	54	85% at 20.9 years
Lopez et al. [107]	80	5.2	88*	25	NA	NA
Cornet et al. [74]	281	4.4	83	18	NA	NA
Hacioglu et al. [108]	78	2.3	81	17	24	96% at 2.1 years
Somasundaram et al. [109]	27	2.2	100	18	48	96% at 2.2 years
Ruiz-Delgado et al. [110]	11	2.1	100	27	NA	91% at 11 years

NA data not available
aafter a second course of cladribine in some patients

cladribine can persist for extended periods with decreases in CD4+ lymphocytes [103]. Herpes zoster is the most frequently reported late infection [75]. Granulocyte colony-stimulating factor (G-CSF) was evaluated in patients treated with cladribine therapy. Although G-CSF ameliorated neutropenia, it did not improve rates of neutropenic fever or hospital admissions for antibiotics and is thus not routinely recommended [114].

Pentostatin (2′-Deoxycoformycin)

Pentostatin is a natural product that is derived from *Streptomyces antibioticus*. Unlike cladribine, it irreversibly inhibits ADA and leads to the accumulation of cytotoxic metabolites. Pentostatin was first described to be an effective agent against HCL in the mid-1980s [115].

One of the largest studies evaluating its efficacy randomized 313 patients to pentostatin or interferon-alpha-2a for 6–12 months. This study used a crossover design where patients in the interferon arm could cross over to pentostatin upon progression. In the initial results, 76% of pentostatin patients achieved a complete remission compared to only 11% treated with interferon-alpha. In patients who crossed over from initial interferon to pentostatin, the complete response rate was 66% [63]. This study underscores the benefits of pentostatin both in treated and untreated patients. Flinn et al. reported on the long-term data from this trial with a median follow-up duration of 9.3 years. The relapse rate was 18% and included both patients initially treated with pentostatin and those who crossed over from the interferon arm. The estimated 5- and 10-year relapse-free survival rates were 85% and 67%, respectively. The 5-year survival was 81%. Acknowledging that this was a crossover design, the survival outcomes were similar between the two groups [116]. The findings in this study have mirrored other long-term follow-up trials with pentostatin (Table 10.3).

Currently, pentostatin is given every 2 weeks usually at a dose of 4 mg/m^2 for 3–6 months until maximum response is achieved. Previous experience with high-dose pentostatin (twice the standard dose) was associated with serious infectious complications [118]. With this interrupted dosing schedule, febrile neutropenia is significantly reduced, especially in comparison to cladribine [63, 71]. Other common side effects of pentostatin include nausea, vomiting, photosensitivity, and keratoconjunctivitis [119].

Cladribine and pentostatin have amassed significant long-term data with many years of follow-up. Else et al. reported on outcomes of 233 patients with a median follow-up of 16 years. In this retrospective review, treatment with single cycle of cladribine or multiple cycles of pentostatin showed equal efficacy: complete remissions (76% vs. 82%) and overall survival (100% vs. 95%) [101]. Lopez et al. described a median treatment-free interval of 95 months with first-line pentostatin and 144 months with first-line cladribine; the difference was not statistically different (p = 0.476) [107]. Despite similar effectiveness, a single course of cladribine is generally considered the preferred first-line treatment because of its brief treatment duration and paucity of adverse effects.

Other Treatments

Splenectomy

Historically, splenectomy was the first effective therapy for HCL. Splenectomy did not affect bone marrow infiltration, but did remove a major site of hairy cell proliferation and alleviated the symptoms of hypersplenism [120]. Most studies noted 60–80% improvement of blood counts with rapid improvements in thrombocytopenia [121, 122]. These responses were not consistent. The degree of splenomegaly was not predictive of hematological improvement or duration of response [123] and most patients ultimately relapsed. The median time to failure with splenectomy was variable, ranging from 5.4 to 56.5 months [124]. No randomized trial has shown a survival benefit with splenectomy [121, 125]. The present indications for splenectomy are active and uncontrolled infection, the resolution of which can be rapid and reflects the improvement of peripheral blood counts. Splenectomy is used in the rare event of a splenic rupture and is beneficial in patients with splenomegaly and severe

Table 10.3 Long-term follow-up studies with pentostatin

Study	Patients (no.)	Patients (no.) with prior therapy	Median F/U (years)	Initial complete remission rate (%)	Relapse rate (%)	Overall survival
Cassileth et al. [117]	50	31	3.25	64	20	NA
Malosiel et al. [65]	238	154	5.3	79	15	89% at 5 years
Flinn et al. [116]	241	87	9.3	71	18	81% at 10 years
Else et al. [101]	188	108	16	82	44	95% at 15 years
Lopez et al. [107]	27	0	12.1	92	51	NA
Cornet [74]	99	0	4.8	82	23	NA

NA data not available

thrombocytopenia who are bleeding. Splenectomy can also be considered in the refractory setting as well as in the second trimester of pregnancy [121, 126].

Interferon

Interferon-alpha is an active agent in HCL and had a significant impact on treatment prior to purine nucleoside analogues. The exact mechanism of action is unknown, but it is thought that interferon-alpha acts as a cytostatic agent in HCL, inducing hairy cell differentiation and making these cells less responsive to growth stimuli [127].

In 1984, Quesada and colleagues first reported the successful use of partially purified alpha human interferon in seven patients with HCL. All seven had normalization of their blood counts with the responses maintained for 6–10 months [128]. Two recombinant interferon-alpha drugs were subsequently developed and approved by the FDA: interferon-alpha-2a and interferon-alpha-2b. Differing by only an amino acid, the recombinant forms showed equal efficacy [129].

Quesada's landmark trial has paved the way to many national and international trials. Treatment with interferon-alpha results mostly in partial remissions ranging from 69 to 87%. Few complete responses are noted and the duration of response is 18–25 months [129–131]. Long-term studies have reported improved survivals of 85–90% at 5 years [132, 133]. Interferon-alpha has activity even in previously treated patients and response rates are robust in patients with splenomegaly [131, 134]. The standard treatment for interferon-alpha-2b is 2×10^6 U/m^2 subcutaneously three times per week for 6–12 months and for interferon-alpha-2a is 3×10^6 U/m^2 three times per week for 12 months. The most common side effects of interferon-alpha are a flu-like syndrome consisting of fever, myalgias, and malaise. Rarely, central and peripheral nervous system complaints have been documented [128, 129].

Although interferon-alpha is an active agent in HCL, it does not induce the same complete responses seen with purine nucleoside analogues and thus is no longer utilized as initial therapy. Treatment with interferon-alpha should be reserved for patients with active infection who cannot receive a purine nucleoside analogue because of its associated immunosuppression.

Evaluation and Follow-Up of Treatment

Patients with HCL should be followed closely for months after treatment to evaluate for cytopenias, possible infectious complications, and ultimately treatment responses [113]. Recovery of blood counts may take weeks to several months following treatment with purine nucleoside analogues. In one study, the median recovery time to normalization of peripheral blood counts after the first cladribine course was 49 days (range 9–379 days) [71]. In addition to evaluating bone marrows for treatment response, translational studies have shown that soluble serum IL-2 secreted by hairy cells correlates closely with disease course and can be used as a noninvasive parameter for disease response [135]. Resolution of hepatosplenomegaly, adenopathy, cytopenias, and eradication of hairy cells from the peripheral blood and bone marrow by non-immunologic studies currently constitutes a complete response [136].

Despite robust responses to treatment with purine nucleoside analogues, long-term studies continue to show late relapses [76, 101]. With this in mind, researchers turned their attention to evaluating minimal residual disease (MRD) in posttreatment bone marrows, in the hopes of more completely eradicating hairy cell infiltrates. MRD can be identified by several techniques: immunohistochemistry using CD20, DBA.44, and CD45RO immunostains; immunophenotyping by flow cytometry; or polymerase chain reaction (PCR) [137–139]. Immunohistochemistry was initially thought to be more sensitive for detecting MRD [43]; however, upon direct comparison, PCR was found to be the most sensitive and specific test [138]. With increased diagnostic sensitivity, these studies have identified residual disease in 10–50% of patients previously thought to be in a complete remission [137, 140] (Table 10.4). This MRD usually represents <1% of the total cell population [137, 141].

Using a strategy of initial cladribine therapy followed by rituximab, researchers have shown that they can successfully eradicate MRD [144, 145]; however, it is not clear if this preemptive treatment strategy translates into improved clinical outcomes. Studies evaluating this question have shown mixed results. Sigal et al. reported on 19 patients who were

Table 10.4 Evaluation of minimal residual disease (MRD)

Study	Method of evaluation	Treatment	Patient (no.)	MRD (%)
Ellison et al. [137]	IHC with anti-CD20 and DBA.44	Cladribine	154	50
Hakimian et al. [140]	IHC with anti-CD20, anti-MB2, anti-UCHL-1	Cladribine	34	21
Wheaton et al. [141]	IHC with anti-CD20 DBA.44, anti-CD45RO	Cladribine	39	13
Matutes et al. [142]	IHC with anti-CD11c, anti-CD25, anti-CD103, and anti-HC2	Pentostatin	31	43
Filleul et al. [143]	PCR, IGH genes	Cladribine	10	100

in complete hematologic remission following initial treatment with cladribine with a median follow-up of 16 years [146]. Using flow cytometry and immunohistochemical staining (CD20, DBA.44, TRAP, and annexin positive), these investigators were able to determine that 47% of patients had no MRD. They also found that in patients with MRD or even gross bone marrow involvement, normal blood counts are possible. The study concluded that HCL is potentially curable and that patients with MRD can have long periods of complete remission [146]. More recently, Lopez et al. reported on a group of 82 patients initially treated with purine analogs and found a shorter treatment-free interval in patients with MRD compared to those without MRD (97 months vs. not reached, $p < 0.049$) [107]. Long-term follow-up with greater number of patients will be needed to fully appreciate the clinical significance of MRD.

Treatment for Relapsed and Resistant Disease

Purine nucleoside analogues elicit noteworthy responses in HCL. Despite this, there are a minority of patients who are refractory to treatment, and their prognosis is inferior. Researchers in Italy investigated biologic parameters in patients who did not respond to cladribine therapy. They found that the unmutated status of IGH variable region paralleled treatment failure and rapid progression of disease. Moreover, they identified defects in TP53 gene as a possible mechanism for resistance. These authors suggested that in such patients, a rituximab-based regimen may be more appropriate [147].

In addition to patients who are resistant to purine nucleoside therapy, long-term follow-up studies suggest that 20–40% of patients who initially had a response will eventually relapse. These patients have several options for therapy when treatment is indicated.

Re-treatment with Purine Nucleoside Analogue

Multiple studies have shown good efficacy when patients are re-treated with purine nucleosides. In the Scripps Clinic series, 62% of patients treated with a second course of cladribine on first relapse achieved a complete remission [71]. Similarly, in the Northwestern experience 83% of relapsed patients responded to a second cycle of cladribine therapy [106]. Even though cladribine and pentostatin have similar chemical structures, there is little clinical cross-resistance between the two drugs [148]. Else et al. showed that relapsed patients had a high rate of remission when treated with the other purine nucleoside analogue. On multivariate analysis, a shorter median duration of first remission was the only variable associated with a failure to attain a complete response [101]. Other studies have noted responses with purine nucleosides in the third- and fourth-line setting, but responses decline with each successive course [149]. No randomized trial exists to determine the optimal duration before re-treatment. Balancing efficacy with immunosuppressive effects of therapy, most experts recommend a 1-year interval before reconsidering purine nucleoside therapy [113].

Immunoconjugates and Targeted Therapies

Rituximab

Because hairy cells express CD20 brightly, rituximab, a chimeric monoclonal antibody against CD20, has become an important agent in salvage therapy. Hagberg first described rituximab as an effective therapy in a patient relapsing from HCL in 1999 [150]. Since then, multiple studies with rituximab have been conducted. Use of single-agent rituximab in the relapsed setting have shown response rates ranging from 25 to 80%, with complete response rates as high at 53% [151, 152]. Given these robust response rates, attention then turned to combination regimens with purine nucleoside analogs. Else and colleagues recently updated their results from a series of 26 patients treated with rituximab and either pentostatin ($n = 15$) or cladribine ($n = 11$) following purine analog treatment failure. Rituximab was administered at a dose of 375 mg/m^2 for 4–8 intravenous infusions (median 6.5). Twenty patients received rituximab concurrently and the remainder received sequential therapy. The overall response rate was 96%, and the complete response rate was 88%. At a median follow-up of 78 months, relapse-free survival following combination therapy was markedly improved compared to the RFS of the same patients following their prior first-line treatment (hazard ratio: 0.10; 95% CI: 0.03–0.32; log-rank $p < 0.0001$). Relapse-free survival at 10 years was 87% (95% CI: 75–100%) following combination therapy, versus 12% (95% CI: 0–24%) following the patient's same first-line treatment [153]. The authors did note certain discrepancies: some patients may have had a heavier baseline disease burden at the time of initial therapy compared to relapse, and 8 of the 26 patients received purine analog in combination therapy that they had not been previously exposed to. Though additional studies of rituximab combination therapy with purine analogs have also shown high response rates for classical [154–156] and variant HCL [157], true randomized trials are lacking.

BRAF Inhibitors

Building on the Italian group's discovery of the BRAF V600E mutation in a very high percentage of classical HCL patients, studies exploring the role of BRAF inhibitors have

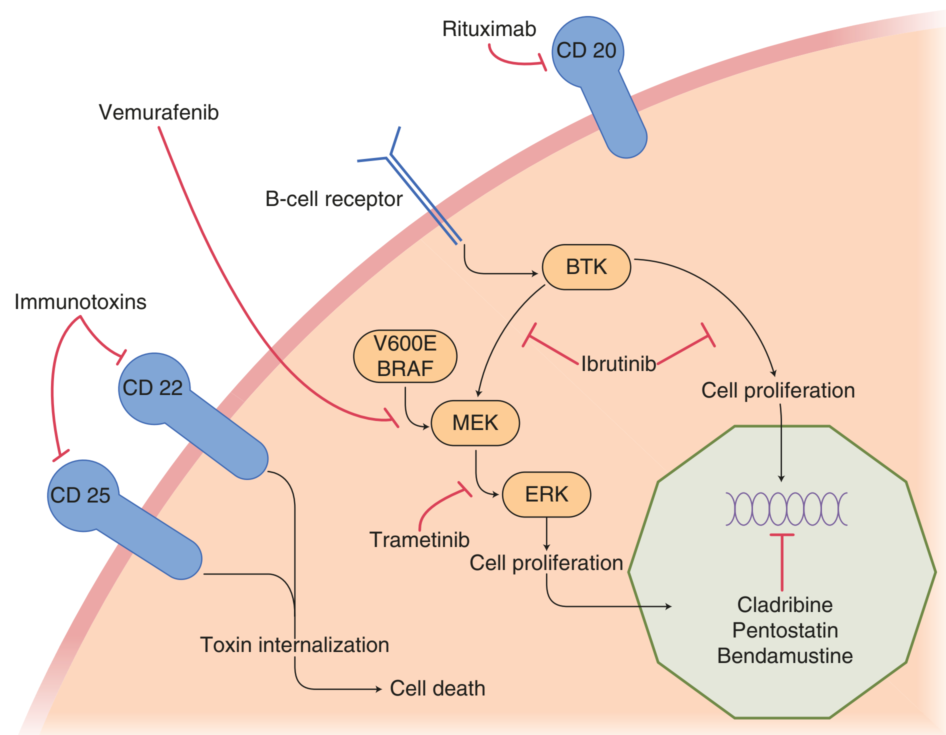

Fig. 10.5 Hairy Cell leukemia therapeutic pathways. *MEK* indicates mitogen-activated protein kinase, *ERK* extracellular signal-regulated kinase, *BTK* Bruton's tyrosine kinase

yielded encouraging results. Early case reports revealed rapid responses, including complete responses, in multiply treated relapsed and refractory classical HCL patients treated with the oral BRAF inhibitor, vemurafenib [158–161] (Fig. 10.5). More recently, results from two phase 2, single-group, multicenter studies of vemurafenib at a dose of 960 mg twice daily were published [162]. The studies were conducted in Italy and the United States. In the Italian study, treatment was administered for a minimum of 8 weeks and, if patients did not have a complete response, for a maximum of 16 weeks. At a median follow-up of 23 months, relapse-free survival was 19 months among patients with a CR. In the US study, patients received treatment on a continuous schedule for 12 weeks; however, patients with residual disease were allowed to receive vemurafenib for up to 12 additional weeks. Median treatment duration was 18 weeks and resulted in an overall survival rate of 91% after a median follow-up of 12 weeks. Therapy was generally well tolerated; drug-related adverse events were mostly grade 1 or 2. Toxicities included rash, arthralgias, and arthritis, among others. However, despite the high response rates, MRD was noted in all patients with complete responses at the end of treatment. In both trials, re-treatment with vemurafenib at relapse elicited some responses.

In the Italian study, bone marrow specimens from 13 of 26 patients were evaluated for phosphorylated ERK and PAX5 double immunostaining. Residual hairy cells expressing ERK were seen in 6 of the 13 patients. All 6 of these patients had a partial response to vemurafenib. Conversely, two patients with complete responses did not express phosphorylated ERK. Post hoc analysis revealed a prolonged median progression-free survival for patients with phosphorylated ERK compared to those lacking this (8 months vs. 13 months, respectively). In addition, residual disease (assessed by the Hairy Cell Index) was greater in patients with persistent phosphorylated ERK than those without measurable ERK. These investigators proposed that circumvention of BRAF inhibition may be explained by alternative mechanisms for reactivating MEK and ERK. The persistence of HCL cells and potential identification of resistance mechanisms indicate the need for additional therapy to improve response rates. One potential method may be to combine a BRAF inhibitor with another drug that targets the pathway, like an MEK inhibitor, as has been done in melanoma patients [163].

MEK Inhibition

The activity of the MEK inhibitor, trametinib, was recently supported by both in vivo and in vitro studies [164]. Pettirossi et al. isolated HCL cells from 26 patients and exposed them in vitro to active BRAF inhibitors (vemurafenib or dabrafenib) or trametinib. The in vitro results were subsequently validated in vivo in the phase 2 study of refractory and relapsed HCL patients treated with oral vemurafenib as detailed above. Vemurafenib, dabrafenib, and trametinib incubation resulted in dose-dependent MEK and ERK

dephosphorylation as well as considerable loss of the hairy morphology. Moreover, the ERK dephosphorylation and apoptosis appeared to be potentiated by the combination of BRAF and MEK blockade with dabrafenib and trametinib. These findings highlight feasible approaches for new treatment options and warrant investigation.

Recombinant Immunotoxins (CD22, CD25)

Recombinant immunotoxins are antibody-toxin chimeric proteins. By engineering the antibody moiety to target antigens expressed preferentially in HCL cells, the toxin moiety is able to exert lethal effects selectively. Recombinant immunotoxin research in HCL has focused on CD25 and CD22.

Kreitman et al. have extensively reported on the efficacy of BL22, a recombinant immunotoxin comprised of a pseudomonas exotoxin fused to a single-chain variable fragment of anti-CD22 [165–168]. Initial results revealed complete remissions in patients with HCL resistant to purine analog therapy [165]. Phase 2 testing of 36 patients with relapsed and refractory HCL, including three patients with variant HCL, was completed [167]. In this study, the complete response rate was 25% after one cycle. Twenty patients were then re-treated, and 47% achieved a complete response. Interestingly, response rates were higher in non-splenectomized patients without massive splenomegaly. Two patients experienced reversible grade 3 hemolytic uremic syndrome, which did not require plasmapheresis. Subsequently, moxetumomab pasudotox, a new recombinant immunotoxin with a 14-fold increased binding affinity for CD22, was developed. Phase 1 testing results are encouraging; the overall response rate was 86% and complete remissions were seen in 46% (13 patients). At 26 months, the median disease-free survival had not been reached. No dose-limiting toxicities were observed [169].

LMB-2 is a recombinant immunotoxin formed from the fusion of a CD25 antibody to the PE38 toxin. Phase 1 testing of this agent in 35 patients with chemotherapy-refractory CD25-expressing hematologic malignancies revealed measurable responses [170]. The most dramatic activity was seen in the HCL cohort. In this group of four patients, one had a complete response, two had partial responses, and the remaining patient had stable disease. Dose-limiting toxicities included reversible cardiomyopathy and transaminitis.

Alemtuzumab

Though it has not been extensively studied, there have been case reports suggesting the activity of alemtuzumab, a humanized monoclonal antibody against CD52, in HCL [171, 172]. Sasaki et al. treated a patient with variant HCL with splenic irradiation followed by alemtuzumab [171]. Splenomegaly resolved, and leukemic cells were eliminated from the peripheral blood by day 12. These reports warrant further investigation into the role of CD52 targeting in the treatment of HCL.

Bendamustine

Bendamustine is a chemotherapeutic agent with features of both alkylators and purine analogs. Following its initial report [173], bendamustine was further studied in relapsed and refractory HCL patients [174]. The results were encouraging; the overall response rate was 100%. At a dose of 90 mg/m^2 on days 1 and 2, for 6 cycles at 4-week intervals, 67% achieved CR, 100% of which were without MRD. At a median follow-up of 31 months, all complete responders with absent MRD remained in CR. Phase 2 trials are currently under way [175].

Ibrutinib

Ibrutinib, a selective and irreversible inhibitor of Bruton's tyrosine kinase (BTK), has activity in multiple low-grade lymphoproliferative disorders including chronic lymphocytic leukemia, mantle cell lymphoma, and Waldenstrom's macroglobulinemia. Recently, Sivina et al. reported their findings showing that BTK protein is expressed in HCL cells, and ibrutinib significantly inhibited HCL cell proliferation, cycling, and survival [176]. Preliminary safety and efficacy data was recently presented [177], and a phase 2 clinical trial is currently under way [178].

Conclusion

Since its initial description more than 50 years ago, the natural history of HCL has been dramatically altered. In the era of Bouroncle, treatment options were few, including splenectomy, and the median survival was only 4 years. Now with purine nucleoside therapies, many patients enjoy long-term remissions frequently surpassing 10 years with good quality of life. The final chapter in this remarkable tale, however, has not been written. Despite excellent responses to the purine nucleoside analogues, cladribine and pentostatin, a small minority of patients will not respond and a proportion who do will eventually relapse. Though these patients respond upon re-treatment, the responses are less rigorous and there is a concern that further therapy will cause long-term immunosuppression. Moreover, disease-free survival curves have failed to show a plateau after 10 years [149]. A few long-term studies, however, have suggested that some patients may be cured. Focusing attention on identifying refractory

patients with HCL through biologic parameters and employing treatment strategies with targeted agents, monoclonal antibodies, and recombinant immunotoxins are currently under investigation. Introduction of BRAF targeting agents represents a major therapeutic advance and addition to the therapeutic armamentarium for patients with refractory or relapsed disease. More research is still needed in understanding the fundamental biology of this disease. Filling in the gaps in our knowledge of the pathophysiology will aid in the development of better treatments with the prospect of more patients enjoying long-term disease-free survival and perhaps even a curative strategy. Also, delving into the biology of HCL will provide insights and potential treatment directions for more common indolent lymphoproliferative diseases.

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