Hairy cell leukemia: an update
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Hairy cell leukemia is an indolent, chronic B-cell lymphoproliferative disorder comprising approximately 2 to 3% of all adult leukemias in the United States. Hairy cells are clonal expansions of mature, activated B-cells. They co-express CD11c, CD19, CD20, CD22, CD25, and CD103. Hairy cells possess clonal immunoglobulin gene rearrangements and express monoclonal surface immunoglobulin of either IgG or multiple heavy-chain isotypes. Treatment of hairy cell leukemia should be considered for symptomatic patients. It is indicated in patients with significant neutropenia, anemia, thrombocytopenia, symptomatic splenomegaly, constitutional symptoms due to hairy cell leukemia, or recurrent serious infections. Many treatments exist, including cladribine, pentostatin, interferon-alpha, splenectomy, rituximab (mabthera), and BL-22 immunotoxin.

Keywords
hairy cell leukemia, biology, clinical features, therapy

Hairy cell leukemia (HCL) is an indolent, chronic, B-cell lymphoproliferative disorder involving the bone marrow and spleen. Reactive marrow fibrosis and peripheral blood cytopenias are commonly found.

It was initially recognized in 1923 by Ewald [1] who described it as leukämische reticuloendotheliose. The first detailed description was by Bouroncle et al. [2] in 1958, who referred to it as leukemic reticuloendotheliosis. In 1966, Schrek and Donnelly [3], were the first to use the descriptive term hairy cell leukemia, highlighting the irregular cytoplasmic projections of the abnormal mononuclear cells observed in the blood and marrow.

HCL is an uncommon disease, accounting for approximately 2 to 3% of all adult leukemias in the United States, which translates into approximately 600 new patients diagnosed in the United States annually. HCL tends to be seen in middle-aged Caucasian males. There is a 4:1 male predominance and a higher incidence in males of Ashkenazi Jewish heritage. Familial cases have been described [4–6]. It is rare in persons of African or Asian descent. The median age at presentation is 52 years, and it has not been described in children or teenagers.

Biology
A number of chromosomal abnormalities have been described in HCL [7]; however, none of them have been consistently seen. Genetic abnormalities described in HCL include monoallelic deletion and mutations of p53 and BCL-6 [8–10]. The clinical significance of these genetic mutations is unclear.

The normal function and exact site of origin in lymphocytic ontogeny of the hairy cell remains unknown, although many characteristics of the elusive cell have been delineated.

Early attempts to classify the neoplastic hairy cell were based on the morphologic appearance of the cells, particularly the generous cytoplasmic domain and often irregular, lobated-appearing nuclei, which were reminiscent of monocytes. In addition to these cytologic similarities with monocytes, early studies showed some functional similarities. Hairy cells were found to have the ability to function as an accessory cell in generating T-lymphocyte colonies in agar culture under phytohaemagglutinin (PHA) stimulation, which is a function similar to that displayed by both normal and neoplastic monocytes.
B-cell maturation, that is, a mature B cell that is not a plasma cell. For example, hairy cells show increased expression of the src proto-oncogene, a feature they share with other B-cell neoplasms representing later stages in B-cell development, whereas expression of this proto-oncogene is low in neoplasms reflecting early and intermediate stages of B-cell maturation (ALL, CLL, LBL) [21]. Also, hairy cells express PCA-1 antigen, which corresponds to later stages of B-cell ontogeny, with coexpression of tartrate-resistant acid phosphatase positivity [22].

However, an odd finding is that hairy cells have been shown to have relatively low levels of somatic hypermutation, an event that is typically associated with T-cell–dependent, antigen-stimulated maturation of B cells within the follicle, occurring after successful IgG class switching and prior to exiting the follicle to live out their lives as plasma cells or memory B cells [23].

In addition to the curious lack of somatic hypermutation, there are other problems with producing an exact match-up between the monocytoid B cell and the neoplastic hairy cell. A close look at peripheralized cells of a monocytoid B-cell lymphoma with progression to large cell lymphoma compared these circulating cells with HCL. The monocytoid cells were tartrate-resistant acid phosphatase (TRAP) negative and expressed IgM heavy chain, in contrast to HCL [24]. Further confounding the issue, there is an identifiable morphologic and immunophenotypic match between the normal reactive monocytoid B cells in their several possible appearances and the neoplasms that arise from them. Reactive monocytoid cells are identified that correspond tightly to the types of cells seen in monocytoid B-cell lymphoma and its large cell variant [25]. In contrast, no such tight linkage has been demonstrated between any normal reactive cell type seen in benign monocytoid B-cell proliferations and neoplastic hairy cells.

Thus, the best current concept of the normal counterpart of the hairy cell is that of a mature B cell that is similar to the monocytoid B cell, but not exactly like it, having an absence of somatic hypermutation and a curious profile of immunoglobulin heavy chain class expression. Finding these elusive cells has been challenging.

Several studies have hinted at the existence of a small population of circulating B cells as a likely candidate. A murine monoclonal antibody, anti-HC2, which reacts with hairy cells, was shown to be positive in 2.2% of normal peripheral blood lymphocytes. These cells were demonstrated to have surface membrane immunoglobulin of the IgG class, and to coexpress an activation antigen. When cell cultures of these B cells were induced to differentiate, the number of HC2-positive cells increased at day 4 or 5 of culture, but then dropped off; and at the time of maximal plasma cell differentiation of the
B cells on day 7 of culture, the HC2-positive cells were no longer present. These data suggest that the normal counterpart may be a pre-plasma cell B cell with activated features [26]. Another study finds that B-ly7 and CD11c, antibodies used to identify HCL, are also present on a very small population of normal B lymphocytes, which may be the normal counterpart of HCL [27]. Finally, an ultrastructural study using immunogold methodology looked carefully at various subpopulations of B cells in normal peripheral blood and found a tiny subset with an immunologic profile similar to hairy cells (DR+ I, FMC7+, aHC1+, aHC2+). Furthermore, their ultrastructural appearance shows a villous outline with variable numbers of polyribosomes scattered throughout the cytoplasm. These features are extremely compatible with the proposed small population of normal circulating B cells that could be the hairy cell of origin [28].

But even if this normal counterpart cell exists, there is still a missing link. For most lymphoid neoplasms, not only is there a known cell type representing the normal counterpart, but there is also a recognized reactive nonneoplastic hyperplasia of that cell type. For example, we recognize the normal monocytoid B cell, benign monocytoid B-cell hyperplasia, and lymphomas composed of monocytoid B cells (marginal zone lymphomas and diffuse large B-cell lymphomas). We recognize the normal benign follicle center cell, benign reactive follicular hyperplasia, and the neoplastic follicle center cells of follicular lymphoma. Where, then, is the benign reactive proliferation of hairy cells?

An interesting contribution to the question is an entity termed “hairy B-cell lymphoproliferative disorder,” which is described as a polyclonal, nonneoplastic proliferation of cells that are virtually identical to one of the variant forms of HCL (HCL-Japanese variant, which is CD11c positive, CD25 negative, and CD103 variable). Hairy B-cell lymphoproliferative disorder is proposed as a nonneoplastic proliferation of the normal cell of origin that gives rise to HCL [29].

In an elegant recent review of the question of the origin of the hairy cell, Burthem et al. [13] make the point that the elusiveness of the normal counterpart may be because it is rare and hard to detect, or alternatively, that there is no true counterpart because the classic features by which we recognize hairy cells are themselves induced by oncogenic transformation. There is a recognized pathway of B-cell maturation that is “T-independent” and produces a cell type that has many features in common with what we know about the hairy cell. For example, this pathway produces B cells that do not develop in follicles but rather are found in the splenic red pulp, like hairy cells [30]. They can undergo Ig class switching in the absence of "T-dependent antigenic stimuli, outside the follicle, and such class switch-
the cytoplasm; these correspond to the ribosomal lamellar complex observed on electron microscopy [39]. Neutropenia and monocytopenia are very common, with more than 80% of patients affected.

Bone marrow
Microscopy of hairy cells in the marrow reveals that they have monotonous round, oval, or spindle-shaped nuclei that are separated by large amounts of pale-staining cytoplasm in a fine fibrillar network, producing the so-called “fried egg” appearance. Bone marrow infiltration with hairy cells may be diffuse or focal and may be difficult to perceive. The marrow may be hypocellular with scant infiltration of hairy cells admixed with residual hematopoietic tissue [38,40]. Marrow aspiration may be difficult secondary to marked reticulin fibrosis.

Spleen
Splenomegaly occurs in approximately 90% of HCL patients [34,35]. The median weight is 1,300 g [41]. Macroscopically, the spleen typically has a dark red, smooth surface. Light microscopy shows that the hairy cells involve the splenic red pulp. Next, atrophy of the white pulp occurs and it is replaced. Additionally, red cell lakes, also known as pseudosinuses, occur. These are blood-filled spaces lined by hairy cells that have disrupted the normal sinus architecture [42].

Cytochemistry, immunophenotype, and immunohistochemistry
Hairy cell cytoplasm contains isoenzyme 5 acid phosphatase, which resists decolorization by tartrate [43,44]; thus, the hairy cell cytoplasm usually stains strongly for TRAP. A TRAP stain that involves at least two cells with more than 40 granules or with many granules masking the nucleus is typically diagnostic of HCL.

Hairy cells express the pan B-cell antigens CD19, CD20, and CD22, but not CD21 [14,45,46]. In addition, hairy cells typically coexpress CD11c, CD25, and CD103 [27]. CD103 has the greatest sensitivity and specificity for HCL. In 26% of HCL cases, there is weak expression of CD10 and in 5% there is weak expression of CD5.

Peripheral blood flow cytometry is a very useful modality in the diagnosis of HCL. In a study by Robbins et al., 92% of 161 HCL patients had circulating hairy cells identified by flow cytometry, while only 80% of the same patients had hairy cells identified by morphology alone [47]. Recently, Tytherleigh et al. reported on their observation that flow cytometry with CD45-PECy5 (Clone J33) has been useful in the detection of HCL [48].

Immunohistochemistry of bone marrow biopsy samples is useful in the detection of minimal residual disease. The majority of antigens used in the detection of circulating hairy cells are destroyed by fixation and standard processing of bone marrow biopsy specimens [49]. However, L26 (CD20 antibody) and DBA.44 can be used to stain hairy cells in routinely processed paraffin sections of bone marrow [50–52]. L26 exhibits membranous staining, and DBA.44 exhibits both membranous and cytoplasmic granular staining.

Therapy, course, and prognosis of hairy cell leukemia
Indications for therapy
Watchful waiting is appropriate for HCL patients who are asymptomatic in whom and significant cytopenias are absent, since early treatment confers neither a survival nor response benefit. Treatment of HCL should be considered for symptomatic patients. Treatment is indicated for patients with significant neutropenia, anemia, thrombocytopenia, symptomatic splenomegaly, constitutional symptoms due to HCL (fevers or night sweats), or recurrent serious infections. Other indications for treatment include leukocytosis with a high proportion of hairy cells (white cell count greater than 20 × 10^9/L), bulky or painful lymphadenopathy, vasculitis, and bony involvement. Table 1 lists the treatment options for HCL patients.

Cladribine
Cladribine [2-chlorodeoxyadenosine (2-CdA)] is a purine nucleoside analog resistant to deamination by the enzyme adenosine deaminase (ADA). Its mechanism of action is shown in Figure 1. It is administered by a continuous intravenous infusion at a dose of 0.09 mg/kg in 500 mL 0.9% NaCl daily over a 7-day period [53]. Adverse events include fever, anemia, thrombocytopenia, and neutropenia [54]. Immunosuppression, as evidenced by lymphocyte subset analysis, is present for approximately 6 to 12 months following a single course of cladribine [55,56]. Infectious complications that have been observed after cladribine therapy include herpes simplex, herpes zoster, cytomegalovirus, *Staphylococcus*, *Cryptococcus*, and *Candida*.

Table 1. Treatment options for hairy cell leukemia

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Table 1. Treatment options for hairy cell leukemia
β-hemolytic *Streptococcus, Streptococcus pneumoniae, Escherichia coli*, *Mycoplasma pneumoniae*, and *Mycobacterium tuberculosis*, and *Mycobacterium chelonei*. Cladribine should probably not be administered to patients with active or uncontrolled infections.

Goodman et al. [57••] recently reported on the outcome data of 209 Scripps Clinic HCL patients who had at least 7 years of follow-up. Patients received cladribine as a 7-day continuous intravenous infusion at a dose of 0.085 to 0.1 mg/kg daily. Of the 207 assessable patients, 196 (95%) achieved a complete response and 11 patients (5%) a partial response following a single course of cladribine. The overall response rate was 100%. The median first response duration for all responders was 98 months. 76 (37%) patients relapsed after their first course of cladribine. The median time to first relapse for all responders was 42 months. 60 patients (79%) received a second course of cladribine after relapsing. Of the 59 assessable patients, 44 (75%) achieved complete responses and 10 (17%) partial responses, while five (8%) failed to respond. The median second response duration for all responders was 35 months. In addition, cladribine was shown to be efficacious in the treatment of HCL patients even after three and four courses. Of the 209 patients who had at least 7 years of follow-up, six (3%) patients died (all complete responders). The overall survival was 97% recorded at 108 months. In the same study, second malignancies in 379 HCL patients were analyzed. 47 patients (22%) developed 58 second malignancies. The observed-to-expected ratio of developing a second malignancy, as compared with the National Cancer Institute Surveillance, Epidemiology, and End Results data [58], was 2.03. Only three patients developed a hematologic malignancy (non-Hodgkin lymphoma) following cladribine therapy. Other second malignancies included non-melanoma skin cancer, melanoma, and prostate cancer. Similar response data has been reported at other centers (Table 2).

Gillis et al. reported on the incidence of hypoplastic/aplastic foci in bone marrow biopsies of HCL patients in remission following cladribine [59]. In their series of 71 posttreatment biopsies from 23 patients, they found that 47 biopsies had 176 hypocellular foci and that 39 biopsies had no evidence of disease. It is interesting to note that 72% of complete blood counts done at the time of biopsy were normal, suggesting that the hypoplastic areas may not be representative of the whole bone marrow.

**Pentostatin**

Pentostatin (2’-deoxycoformycin) is a tight-binding inhibitor of ADA and hence simulates genetic ADA deficiency, thereby leading to lymphocyte death [60].

Pentostatin is given at a dose of 4 mg/m² every other week, for 3 to 6 months, to maximal response. Adverse effects from pentostatin include fever, nausea, vomiting, photosensitivity and keratoconjunctivitis [61,62]. It is immunosuppressive, and patients with poor bone marrow reserve are at greatest risk [63,64]. Infectious complications that have been observed after pentostatin

| Table 2. Response of hairy cell leukemia to a single course of cladribine |
|-----------------------------|----------------|-----------------|
| Institution                | Number of assessable patients | Complete response rate, % | Partial response rate, % |
| Huddinge and Karolinska Hospitals [84] | 16 | 75 | 0 |
| Long Island Jewish Medical Center [85] | 49 | 76 | 24 |
| MD Anderson Cancer Center [86] | 46 | 78 | 11 |
| National Cancer Institute [87] | 861 | 50 | 37 |
| Northwestern University [88] | 50 | 80 | 18 |
| Royal Marsden Hospital [89] | 45 | 84 | 16 |
| Scripps Clinic [57••] | 207 | 95 | 5 |
therapy include disseminated herpes zoster, *Escherichia coli*, *Hemophilus influenzae*, and pneumococcal and fungal infections [62]. Pentostatin should probably not be administered to patients with active or uncontrolled infections, a poor performance status, or renal dysfunction [63,65].

Flinn et al. [66] reported on the long-term follow-up of HCL patients treated with pentostatin. This was an intergroup phase-3 study that used a randomized crossover design with pentostatin and interferon-alpha-2a. 241 patients were either treated with pentostatin as initial therapy or were treated with pentostatin after failing interferon-alpha. The median duration of follow-up was 9.3 years. The complete response rate was 72%, the 5-year survival rate was 90%, and the 10-year survival rate was 81%. 39 patients developed 44 second malignancies (8 hematopoietic).

Kraut et al. [67] reported on a small cohort of 24 HCL patients who achieved a complete response with pentostatin. The median follow-up was 82 months. 23 patients were alive at a median time of 84 months after achieving a complete response. One patient died of refractory HCL. Of the 23 patients, 11 relapsed at a median of 30 months. Seven patients were retreated after their relapse, four with pentostatin, and three with cladribine. Of the four patients retreated with pentostatin, three achieved a complete response. Of the three patients retreated with cladribine, two patients achieved a complete response, and one patient achieved a clinical complete response. There were three second malignancies (one stage IA Hodgkin at 70 months, one basal cell carcinoma, and one squamous cell carcinoma).

There appears to be a lack of cross resistance between cladribine and pentostatin despite structural and mechanistic similarities. There are reports of patients who have responded to pentostatin after having relapsed after cladribine therapy, and vice versa [54,68]. In a study by Saven *et al.* [54] 358 HCL patients were treated with cladribine; of seven patients salvaged with pentostatin following relapse, three had complete responses, three partial responses, and one patient failed to respond.

Pentostatin may represent a reasonable salvage therapy in selected HCL patients who have failed cladribine, however, there is a paucity of data reflecting the response rates and duration of response in large numbers of patients. Direct comparisons cannot be made between the pentostatin and cladribine trials due to differences in study design. No head-to-head randomized trials have thus far been performed or planned.

**Interferon-alpha**

Interferon-alpha induces partial responses in most HCL patients but complete responses in only the minority of patients [69]. It is useful in the treatment of HCL patients who present with active infections and are therefore unable to undergo purine nucleoside analogue therapy due to the resultant T-cell immunosuppression [33,70]. Interferon-alpha is also useful in patients who have failed purine analogue therapy [71]. A recent in vitro study [72•] showed that therapeutic concentrations of interferon-alpha induced apoptosis of nonadherent hairy cells by increasing the secretion of tumor necrosis factor-alpha and the sensitization of hairy cells to the proapoptotic effect of autocrine tumor necrosis factor-alpha.

Interferon-alpha-2b is given at a dose of 2 million units/m² by subcutaneous injection three times a week for 12 months. Interferon-alpha-2a is initially given at a dose of 3 million units/m² by subcutaneous injection daily for 6 months and then decreased to three times per week for an additional 6 months.

Side effects of interferon-alpha include a flu-like syndrome (fever, myalgias, and malaise). Acetaminophen can be used to ameliorate these symptoms and tachyphylaxis usually develops over time.

In a study by Saven *et al.* [54] 358 HCL patients were treated with cladribine. A total of nine patients were salvaged with interferon-alpha following relapse. Of these nine patients, there was one complete response, two partial responses, and six patients did not respond.

Seymour *et al.* [71] treated 46 HCL patients with cladribine. 41 patients responded; of these, eight patients relapsed. Three of these patients were treated with interferon-alpha, and all three had an objective response, which was maintained while receiving interferon-alpha. However, two patients relapsed after the interferon-alpha was discontinued.

An unexpectedly high incidence of second malignancies has been seen in HCL patients treated with interferon-alpha [73,74]. 69 patients were followed for a median of 91 months. 13 patients (19%) developed a second malignancy; six were hematopoietic, and seven were adenocarcinomas. The median survival was 9 months after diagnosis of a second malignancy. However, the risk of second malignancies from interferon-alpha and/or HCL itself has been disputed. Federico *et al.* [75] reported on 1,022 Italian HCL patients. There were 54 cases of second malignancies in 54 patients. The cumulative risk of developing a second malignancy was 5% at 5 years, 10% at 10 years, and 14% at 15 years. They found that the incidence of second malignancies was not statistically significantly higher than that expected from the multiple Italian cancer registries. They did note, however, that the incidence of lymphoid neoplasms was higher than expected.
Splenectomy

Splenectomy was the first standard treatment used in HCL. It affords rapid relief in peripheral blood cytopenias. Post-splenectomy, approximately 40 to 60% of patients will normalize their blood counts, and 90% will have an improvement in at least one hematologic parameter [76, 77]. Current indications for splenectomy include active or uncontrolled infections, bleeding associated with severe thrombocytopenia, massive painful or ruptured splenomegaly, and chemotherapy failure, including cladribine. Response to splenectomy is not uniform, since cytopenias may be due to diffuse bone marrow infiltration by HCL rather than principally hypersplenism from splenic enlargement [78••].

Rituximab (mabthera)

Hairy cells express the pan B-cell antigens, including CD20. Rituximab, a monoclonal antibody targeting CD20, has been studied in refractory or relapsed HCL. Thomas et al. [79] reported on a MD Anderson Cancer Center study using rituximab in eight refractory HCL patients. Of the five assessable patients, four patients responded (two complete responses, one complete response with minimal residual disease, and one partial response). The toxicity reported was limited to rigors associated with the rituximab infusion in seven of the eight patients. Nieva et al. [80••] from Scripps Clinic reported on a phase II study of rituximab in cladribine-failed HCL. Of 15 assessable patients, there were two complete responses, one complete response with minimal residual disease, and two partial responses. Rituximab therapy was well tolerated.

Hagberg et al. [81••] reported on a Swedish study of eight relapsed and three newly diagnosed HCL patients treated with rituximab. The patients were given rituximab 375 mg/m² once a week for 4 weeks. Seven of 11 (64%) responded with six complete remissions and one partial remission. Remissions lasted for a median of 14 months. One of the three newly diagnosed HCL patients had a complete response, and the other two had stable disease.

BL-22 Immunotoxin

Kreitman et al. [82••], in a recent article, demonstrated the efficacy of a recombinant immunotoxin BL-22 in the treatment of HCL. BL-22 contains the variable domain of an anti-CD22 monoclonal antibody fused to a fragment of a Pseudomonas exotoxin. In this small study of 16 cladribine-resistant patients, 11 achieved a complete response and two a partial response. Two patients developed reversible hemolytic-uremic syndrome. Although BL-22 represents an exciting new approach to the treatment of this disease, these results need to be interpreted cautiously, given the potential life-threatening toxicity of this agent.

Summary

HCL is an indolent, chronic B-cell lymphoproliferative disorder comprising approximately 2 to 3% of all adult leukemias in the United States. Hairy cells are clonal expansions of mature activated B-cells. They coexpress CD11c, CD19, CD20, CD22, CD25 and CD103. Watchful waiting is appropriate for HCL patients who are asymptomatic. Treatment is indicated in patients with significant neutropenia, anemia, thrombocytopenia, symptomatic splenomegaly, and constitutional symptoms due to HCL or recurrent serious infections. Many treatments exist including cladribine, pentostatin, interferon-alpha, splenectomy, rituximab (mabthera), and BL-22 immunotoxin. Survival has been significantly extended with the usage of purine analog therapy.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- Of special interest
- Of outstanding interest


This article provides one mechanism by which interferon-alpha induces responses in hairy cell leukemia.


This is a comprehensive review of the treatment of hairy cell leukemia.


This is an early report on the efficacy of rituximab (mabthera) in the treatment of hairy cell leukemia.


This is an early report on the efficacy of rituximab (mabthera) in the treatment of hairy cell leukemia.


This is an early report on the efficacy of, and the adverse events from, BL-22 in the treatment of hairy cell leukemia.


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