Update on Hairy Cell Leukemia

Robert J. Kreitman, MD, and Evgeny Arons, PhD

The authors are affiliated with the National Cancer Institute's Center for Cancer Research at the National Institutes of Health in Bethesda, Maryland. Dr Kreitman is a senior investigator in the Laboratory of Molecular Biology and the head of the clinical immunotherapy section, and Dr Arons is a staff scientist in the Laboratory of Molecular Biology.

Corresponding author:
Robert J. Kreitman, MD
Laboratory of Molecular Biology
National Cancer Institute
National Institutes of Health
9000 Rockville Pike
Building 37, Room 5124b
Bethesda, MD 20892-4255
Tel: (301) 648-7375
Fax: (301) 451-5765
E-mail: kreitmar@mail.nih.gov

Keywords
Bendamustine, cladribine, dabrafenib, ibrutinib, moxetumomab pasudotox, pentostatin, rituximab, trametinib, vemurafenib

Abstract: Hairy cell leukemia (HCL) is a chronic B-cell malignancy with multiple treatment options, including several that are investigational. Patients present with pancytopenia and splenomegaly, owing to the infiltration of leukemic cells expressing CD22, CD25, CD20, CD103, tartrate-resistant acid phosphatase (TRAP), annexin A1 (ANXA1), and the *BRAF* V600E mutation. A variant lacking CD25, ANXA1, TRAP, and the *BRAF* V600E mutation, called HCLv, is more aggressive and is classified as a separate disease. A molecularly defined variant expressing unmutated immunoglobulin heavy variable 4-34 (IGHV4-34) is also aggressive, lacks the *BRAF* V600E mutation, and has a phenotype of HCL or HCLv. The standard first-line treatment, which has remained unchanged for the past 25 to 30 years, is single-agent therapy with a purine analogue, either cladribine or pentostatin. This approach produces a high rate of complete remission. Residual traces of HCL cells, referred to as minimal residual disease, are present in most patients and cause frequent relapse. Repeated treatment with a purine analogue can restore remission, but at decreasing rates and with increasing cumulative toxicity. Rituximab has limited activity as a single agent but achieves high complete remission rates without minimal residual disease when combined with purine analogues, albeit with chemotherapy-associated toxicity. Investigational nonchemotherapy options include moxetumomab pasudotox, which targets CD22; vemurafenib or dabrafenib, each of which targets the BRAF V600E protein; trametinib, which targets mitogen-activated protein kinase enzyme (MEK); and ibrutinib, which targets Bruton tyrosine kinase (BTK).

Introduction

Hairy cell leukemia (HCL) was reported in 1958 by Bouroncle and colleagues as an indolent malignancy associated with pancytopenia and splenomegaly that accounted for 2% of all leukemias.[1] On the basis of the projected 2017 US incidence of leukemia of 62,130 cases,[2] a 2% incidence translates to approximately 1240 new cases of HCL occurring per year. Early treatments included corticosteroids, nitrogen mustard and other alkylators, splenic radiation, and

splenectomy.[3] By 1978, the median overall survival was approximately 4 years.[4] The first effective systemic therapy was interferon, which produced a median disease-free survival of up to 3 years.[5] Interferon is still advocated today in limited circumstances.[6] A major leap forward was the development of purine analogues. This includes pentostatin (also called 2-deoxycoformycin, or DCF), with results reported in 1986 and 1987,[7,8] and cladribine (also called 2-chlorodeoxyadenosine, or CDA), with results reported in 1990.[9] A variant of HCL, called HCLv, was reported in 1980[10,11] and accounts for 10% to 20% of patients with HCL. In the past 10 to 15 years, progress has been made in nonchemotherapy approaches to HCL, including monoclonal antibody (MAb)–based immunotherapy (recombinant immunotoxins) and agents that target BRAF. This review provides an update to the diagnosis and treatment of HCL, particularly with nonchemotherapy approaches.

Diagnosis of Hairy Cell Leukemia

The median age of patients who present with HCL is approximately 55 years, although cytopenias lasting for years can precede the diagnosis.[12] The male-to-female ratio is approximately 4:5, and the incidence is elevated among whites and reduced among Asians, Arabs, and Africans.[12] Class II human leukocyte antigen (HLA) DRB1*11 is overexpressed in white patients who have HCL compared with those who do not have HCL, and expression is higher in whites than in other populations.[13] Environmental risk factors include farming, exposure to pesticides and other chemicals, and ionizing radiation, but not tobacco use.[12] Patients who have HCL commonly present with fatigue (80%), fevers, night sweats, infections, weight loss, and left upper abdominal pain and bruising.[14] Blood or bone marrow aspirate flow cytometry shows bright positivity for CD22, CD20, and CD11c, and positivity for CD19, CD103 and CD123.[14,15] CD25 may be bright to dim. Bone marrow biopsy immunohistochemistry (IHC) stains CD20, tartrate-resistant acid phosphatase (TRAP), annexin 1A (ANXA1), DBA44 (CD72), and the *BRAF* V600E mutation.[14,16,17]

Diagnosis of Variants of Classic Hairy Cell Leukemia

A more aggressive variant of HCL lacking CD25, ANXA1, TRAP, and BRAF V600E, called HCLv, has been classified as a separate disease by the World Health Organization.[10,11,18] HCLv responds poorly to purine analogues when they are used alone, but much better when they are combined with rituximab (Rituxan, Genentech/Biogen Idec).[19,20] HCLv has more lymphocytosis and less severe cytopenias compared with classic HCL.[11] Flow cytometry shows that bright B-cell antigens and CD11c, but not CD25 and CD123, are associated with classic HCL.[11,15] Positivity for CD103 is nearly always present in HCLv and HCL, and its absence should suggest splenic marginal zone lymphoma.[15,21] In both HCL and HCLv, the red pulp of the spleen is infiltrated, whereas in splenic marginal zone lymphoma, the white pulp is infiltrated.[11,17] HCLv differs from HCL in that the intrasinusoidal bone marrow is involved.[11,17] HCLv shares features with splenic diffuse red pulp lymphoma (SDRPL), and both HCLv and SDRPL fall within the category of "splenic B-cell lymphoma/leukemia, unclassifiable" in the World Health Organization Classification of Tumours.[11] Compared with HCLv, SDRPL is associated with less lymphocytosis and anemia, longer overall survival after diagnosis, lower CD11c and CD103 expression, and higher CD123 expression.[11] A variant expressing unmutated immunoglobulin rearrangement IGHV4-34 is also aggressive and lacks BRAF V600E but has an immunophenotype of HCL or HCLv.[18,22,23] Patients with IGHV4-34–positive HCL that resembles classic HCL immunophenotypically, including bright CD25 positivity, can have clinical features of HCLv, including lymphocytosis, and massive splenomegaly with nodal disease.[22,24] Classic HCL can express unusual markers. CD38 is associated with a shorter mean time to salvage therapy, and in mice, CD38 may promote HCL cell survival and adhesion.[25] CD5 expression in HCL is rare, is reported more commonly in HCLv, and may not be related to prognosis.[26] CD10, expressed in 14% of cases of HCL, was reported in a patient who had HCL with nodal involvement, which is variably linked to advanced disease and is itself a poor risk factor,[27,28] but CD10 positivity has not been clearly linked to HCL prognosis.[29] Finally, the Japanese variant of HCL (HCLjv) has a poor response to single-agent purine analogues and a better response to rituximab. HCLjv occurs in Japanese men and features lymphocytosis, negativity for CD25 and ANXA1, and variable CD103 expression. HCLjv cells are usually CD27-negative, although CD27-positive HCLjv was recently reported.[30]

Indications for the Treatment of Hairy Cell Leukemia

The most common indication for treatment has been the presence of at least one cytopenia, defined as a neutrophil count of less than 1 to 1.5/nL, a hemoglobin level of less than 10 to 12 g/dL, or a platelet count of less than 100/nL.[31-33] To avoid excessively rapid re-treatment, protocols from the National Cancer Institute and Dr Alan Saven's group generally use the more stringent criterion of a neutrophil count of less than 1/nL, a hemoglobin

Table. Agents and Regimens Recently Tested in HCL/HCLv

Agent(s)	Route	Disease	Eligibility	Target	Includes Chemo?
Cladribine, SC	SC	HCL	No prior cytostatic treatment	–	Yes
Cladribine/rituximab	IV	HCL/HCLv	0-1 prior purine analogue	CD20	Yes
Bendamustine/rituximab vs pentostatin/rituximab	IV	HCL	≥2 prior treatments	CD20	Yes
		HCLv	0 or more prior treatments		
Moxetumomab pasudotox	IV	HCL/HCLv	≥2 prior treatments	CD22	No
Ibrutinib	Oral	HCL	≥1 prior treatment	BTK	No
		HCLv	0 or more prior treatments		
Vemurafenib	Oral	HCL	≥1 prior treatment	BRAF	No
Dabrafenib/trametinib	Oral	HCL	≥2 prior treatments	BRAF/MEK	No

BTK, Bruton tyrosine kinase; chemo, chemotherapy; HCL, hairy cell leukemia; HCLv, HCL variant; IV, intravenous; MEK, mitogen-activated protein kinase enzyme; SC, subcutaneous.

level of less than 10 g/dL, or a platelet count of less than 100/nL.[32,34-39] Additional eligibility criteria have included malignant lymphocytosis (>5 or 20/nL), splenomegaly, enlargement of malignant lymph nodes, and frequent infections. These additional criteria are important for patients with HCL following splenectomy and for those with HCLv because these patients typically lack cytopenias yet have more advanced disease. Before previously treated patients are re-treated, it is important to determine whether cytopenias are due to chemotherapy toxicity or to relapsed or refractory HCL. The bone marrow biopsy specimen may show significant infiltration of HCL 1 to 3 months after purine analogue therapy, but then be consistent with complete remission (CR) by 6 months without intervening therapy. Thus, new approaches to the treatment of relapsed or refractory HCL should begin only when restaging has been performed at a sufficiently long interval (4-6 months) after the prior therapy. Flow cytometry of blood showing an increase in circulating HCL cells often helps to confirm that HCL remaining after prior therapy will not resolve on its own.

Purine Analogues, the Standard First-Line Treatment of Hairy Cell Leukemia

The treatment of HCL was revolutionized by the purine analogues pentostatin and cladribine (Table), which achieved CR rates of 70% to 90% and treatment-free intervals exceeding 10 years.[31,32,40,41] Pentostatin is given every other week for 3 to 12 months, whereas cladribine is given as a single 5- to 7-day course. The toxicities of pentostatin and cladribine are similar; they include neutropenia and fever (particularly during the first month), CD4-positive T-cell reductions lasting 40 to 52 months,[42,43] and long-term neuropathy.[32,44] Because the results are so similar with the 2 agents[45] and cladribine has a more convenient schedule, cladribine is the more commonly used purine analogue by far.

In patients who are followed with blood cell counts and in whom the need for additional treatment arises, the median time to relapse after the first-line use of a purine analogue is approximately 16 years.[40] When a population of young patients (≤40 years of age at diagnosis) was followed with bone marrow biopsy, the median time to relapse from CR was less than 5 years,[46] although many patients did not require re-treatment at the time of relapse. It is not clear whether relapse occurs earlier in younger than in older patients or whether younger patients are more likely to receive frequent follow-up care, including frequent and intensive re-staging. The time between first and second therapy is shorter in patients who are younger than 40 years at diagnosis than in older patients (63 vs 145 months; P=.008), and they receive more lines of therapy during follow-up.[47] Long-term failure-free follow-up curves fail to show a plateau indicating a population with cure,[40,45] and it is unknown what percentage of patients, if any, have disease cured by purine analogues. Of the original 358-patient cohort treated with cladribine at the Scripps Clinic, 9 of 19 in continuous CR for a median of 16 years were free of HCL MRD by flow cytometry and IHC. This suggests that the disease of at least some patients may be cured. Nevertheless, relapse in HCL is common and has resulted in an accumulation of patients with relapse who require additional options.

Criteria for Response Reflecting the Goals of Treatment

Criteria for CR include the elimination of HCL as determined by nonimmunologic staining, including staining

of blood and bone marrow aspirate with Wright stain, and staining of bone marrow biopsy specimens with hematoxylin and eosin. Additional criteria include the resolution of organomegaly on examination, adenopathy (short axis ≤2 cm), and cytopenias to a neutrophil count of at least 1.5/nL; hemoglobin levels of at least 11 to 12 g/dL; and platelet levels of at least 100/nL.[33-39,44,48] If imaging is used, the long axis of the spleen either should measure no more than 17 cm or should be decreased more than 25% from baseline. We have dropped the hemoglobin requirement in some protocols when iron deficiency is documented. Partial remission (PR) criteria vary, but we and others require either fulfillment of CR criteria or at least a 50% reduction in palpable hepatosplenomegaly, number of circulating HCL cells, sum of lymph node perpendicular diameters, and at least a 50% improvement in cytopenias.[32,34-39,44,49] PR with the resolution of normal counts to CR levels is often considered a good PR (GPR), or hematologic remission. For patients receiving blood transfusions at baseline, we accept a post-treatment hemoglobin level of 9.0 g/dL as consistent with PR. A CR or PR cannot be documented until 4 weeks after the last transfusion or dose of growth factor (granulocyte colony–stimulating factor, granulocyte-macrophage colony–stimulating factor, or erythropoietin). In addition, we and others require resolved or at least 50% improved normal blood cell counts lasting at least 4 weeks for PR or CR to be considered.[31,44] However, in early HCL, response may be determined on the basis of a single assessment[32] because after first-line treatment, a patient with a resolved complete blood cell count may not have to be retested for many months. Cytopenias following treatment for HCL can be due to prolonged toxicity, so patients without MRD—including those with negative results of bone marrow aspirate flow cytometry—are considered to be in CR even if cytopenias are present. However, residual adenopathy can represent residual HCL in patients who are otherwise without MRD; lymph node biopsy may be required to determine response in these patients.

Complete Remission With or Without Minimal Residual Disease

MRD may be detectable in patients in CR by several methods, including flow cytometry of blood or bone marrow, or IHC of a bone marrow biopsy specimen. Of these tests, flow cytometry of bone marrow aspirate is by far the most sensitive.[20,50] Flow cytometry must be done with multiple colors and should be able to detect a level of HCL cells of less than 0.01%. The criteria for MRD positivity by IHC include the presence of CD20-positive or DBA44-positive cells that are mostly consistent with HCL morphologically, and levels of B cells that are at least as high as those of T cells.[51] A more sensitive test of HCL MRD has been developed in which a sequence-specific probe and primers are used to detect the unique immunoglobulin rearrangement (immunoglobulin heavy locus), which is generally identical for all HCL cells from each patient and should differ from one patient with HCL to another. This real-time quantitative polymerase chain reaction (PCR) test can detect a level of HCL cells as low as 1 HCL cell per 10^6 normal cells,[52] but each patient requires a special PCR assay, so this test is currently considered investigational.

Treatment With Single-Agent Rituximab

Rituximab kills CD20-positive cells by inducing apoptosis and mediating either complement-dependent cytotoxicity or antibody-dependent cell-mediated cytotoxicity.[53] CR rates vary, but in the largest trial—of 24 patients who required treatment for cytopenias and had previously been treated with at least one purine analogue[54]—researchers observed 3 CRs (13%) and 3 PRs (13%). The most common toxicity in HCL is fever and rigors, although rare severe toxicity has been reported.

Treatment With the Combination of Rituximab and a Purine Analogue for Hairy Cell Leukemia

Retrospective data on 26 patients who had received 1 to 6 (median, 3) prior courses of a purine analogue and were treated with rituximab plus either cladribine (11 cases) or pentostatin (15 cases) were recently updated.[45] Among 25 evaluable patients, the CR rate was 88% and the overall response rate (ORR) was 96%. Relapse-free survival was significantly longer with rituximab/purine analogue than with previous single-agent purine analogue treatment (hazard ratio [HR], 0.1; $P<.0001$). This improvement in relapse-free survival was attributed to the use of rituximab, the use of a different purine analogue, and the patients' smaller disease burden compared with that before first-line treatment.[45] A prospective trial of cladribine and rituximab (CDAR) in HCL, in which patients received 5 daily doses of cladribine (0.15 mg/kg) followed 1 month later by 8 weekly doses of rituximab (375 mg/m^2), was recently updated. Of 59 patients with newly diagnosed disease, all had a CR. After a median follow-up of 50 months (range, 3-128), only 1 patient had experienced a relapse and 1 patient had died of a second malignancy.[55] MRD positivity by flow cytometry of the blood and/or bone marrow was found in 6 of 28 patients (21%) 3 months after they had started CDAR.[19] Of 14 patients who had once-relapsed HCL treated with CDAR, all had a CR, and the CR after CDAR was longer than the CR in the same patients after

first-line treatment with a purine analogue.[55] In 2009, we initiated a randomized trial of CDAR for both untreated and once-relapsed HCL, at the same doses as those used in the trial by Ravandi and colleagues. Patients were randomly assigned to 8 weekly doses of rituximab, begun either on the same day as cladribine or at least 6 months after cladribine if and when MRD was observed in the blood. This regimen took advantage of the synergy of concurrent therapy, whereby rituximab renders malignant cells more sensitive to the purine analogue.[56] In 2010, we initiated a randomized trial in patients with multiply relapsed HCL, in which we examined the concurrent administration of rituximab with either bendamustine (Treanda, Bendeka; Teva) or pentostatin. To determine the appropriate dose of bendamustine, 2 groups, each including 6 patients, received bendamustine/rituximab (BR) treatment; bendamustine was administered at a dose of either 70 or 90 mg/m^2 on days 1 and 2 of 6 cycles 28 days apart, and rituximab was administered at a dose of 375/m^2 on days 1 and 15.[50] All 12 patients responded, with CRs observed in 4 of the 6 patients who received bendamustine at 90 mg/m^2 vs 3 of the 6 patients who received bendamustine at 70 mg/m^2. Both the rates of MRD-negative CR (67% vs 33%) and the times to CR (111 vs 223 days) favored the higher dose of bendamustine, with a trend (P=.057) toward significance for time to CR. This trial is currently randomizing patients either to rituximab/pentostatin or to rituximab plus the higher dose of bendamustine. Thus, combinations of rituximab and purine analogues are highly effective in achieving CR and eradicating MRD, although with immunosuppressive toxicities due to the purine analogue.

Combination Rituximab and Cladribine for Early-Stage Hairy Cell Leukemia Variant

Ravandi and colleagues treated 5 patients with HCLv,[19] with a CR achieved in all of them. Of these, 3 patients died—1 of relapse and 2 of secondary malignancies. To determine the effect of synergy between rituximab and cladribine in HCLv, we treated patients who had HCLv with concurrent CDAR; the first 8 of weekly doses of rituximab were begun on day 1 of the 5 daily doses of cladribine,[20] as in our CDAR trial for early HCL. In HCLv, we reported a CR rate of 90% and an MRD-negative CR rate of 80% in 10 patients with either newly diagnosed or once-relapsed disease after cladribine. As in the previously discussed BR trial,[50] we reported transient thrombocytopenia with the concurrent use of a purine analogue and rituximab for HCL; the median drop in the platelet count was 50/nL. The 90% CR rate was superior to the less than 10% CR rate in 39 previously reported historical patients (P<.0001). Thus, cladribine alone should no longer be considered appropriate standard care for HCLv, and we believe these patients should receive combination treatment with a purine analogue plus rituximab or investigational therapy. Patients who have HCLv are also eligible to receive BR or pentostatin/rituximab (DCFR) on trial, regardless of prior treatment.

Recombinant Immunotoxins: An Introduction

Protein (either plant or bacterial) toxins are highly potent catalytic agents capable of killing a cell with a single molecule in the cytosol. Targeted protein toxin immunoconjugates were made smaller and less expensive by using fusion toxins containing a cell-binding ligand and a truncated protein toxin (Figure 1). The protein toxin portion of the fusion toxin lacks the toxin domain binding to normal animal cells. Recombinant immunotoxins are fusion toxins in which the ligand is a recombinant variable fragment (Fv) of a MAb. The earliest recombinant immunotoxin contained a single-chain Fv of the anti-CD25 (anti–interleukin 2-receptor α) MAb anti-Tac fused to the 40-kDa fragment of the *Pseudomonas* exotoxin, which was without its binding domain.[57] This molecule was called anti-Tac(Fv)-PE40. Anti-Tac(Fv)-PE38, containing a 38- rather than a 40-kDa truncated toxin fragment, was produced for clinical trials under the name LMB-2.[58,59]

LMB-2 for Hairy Cell Leukemia

The development of an anti-CD25 recombinant immunotoxin, which led to the formulation of LMB-2, was originally aimed at adult T-cell leukemia[35,60,61] in addition to other leukemias and lymphomas, including chronic lymphocytic leukemia.[60] In preclinical studies, LMB-2 was found to be highly cytotoxic ex vivo to freshly obtained HCL cells.[62] In the phase 1 trial of LMB-2 in a variety of hematologic malignancies, this agent was tested in 4 patients with HCL, resulting in 1 CR and 3 PRs.[34,35] The complete responder received 2 cycles of LMB-2, each given every other day for a total of 3 doses (QOD × 3), and this patient did not require additional therapy until nearly 8 years later. Responses to LMB-2 were also seen in other leukemias and lymphomas.[35] Because many of these hematologic malignancies, including HCL/HCLv, were more often and more strongly CD22-positive than CD25-positive, immunotoxin treatment of HCL was shifted to target CD22.

Targeting CD22 With BL22 in Hairy Cell Leukemia

Chemical conjugates of anti-CD22 antibodies—including RFB4—and deglycosylated ricin A chain (dgA) have been found to be cytotoxic to malignant B cells. Furthermore,

RFB4-dgA has shown clinical activity against B-cell leukemias and lymphomas.[63] Chemical immunoconjugates have been made by conjugating LL2 or RFB4, both of which are anti-CD22 MAbs, and truncated *Pseudomonas* exotoxin.[64,65] RFB4 proved to be optimal for making a cytotoxic recombinant immunotoxin.[66] BL22, shown in Figure 1, contained a PE38 toxin fragment (as did LMB-2), but the Fv was disulfide-stabilized by mutation of both Arg44 of VH and Gly100 of VL to cysteine. These 2 residues were predicted to be approximately 5.5 angstroms apart, and the disulfide bond formed during in vitro renaturing of the diluted and reduced *Escherichia coli* inclusion body protein in redox buffer.[66,67] Thus, BL22 is considered recombinant because it forms during renaturation without the need for chemical conjugation. BL22 was cytotoxic to CD22-positive leukemic cells ex vivo[68] at concentrations achievable in monkeys.[69] In phase 1 testing of BL22, CRs were achieved in 19 of 31 (61%) and PRs in 6 of 31 (19%) patients with relapsed or refractory HCL, including 3 patients with HCLv.[36,37] Dose-limiting capillary leak syndrome, a common toxicity of immunotoxins attributed to endothelial damage, was not observed in these patients, except in 1 case in association with cytokine release syndrome. Instead, a completely reversible hemolytic uremic syndrome, characterized by transient thrombocytopenia, hemolytic anemia, and renal dysfunction, was observed in 13% of patients.[37] Only 1 patient in this trial was permitted by protocol to receive enough cycles to eradicate MRD by bone marrow aspirate flow cytometry, and this patient has been in a continuous MRD-negative CR for 14 years. In a phase 2 trial of 36 patients, the CR rate was 47% and the ORR was 72%, with 2 patients remaining MRD-negative for 10 to 12.5 years.[38] Future development of this agent continued with a higher-affinity mutant to allow more selective binding to CD22.

Development of Moxetumomab Pasudotox for Hairy Cell Leukemia

To increase its affinity and selectivity for CD22, phage selection was used to select random mutations within "hot spots" of the RFB4 VH third complementarity-determining region (CDR3) domain. This process isolated a mutant containing amino acids threonine, histidine, and tryptophan (THW) instead of serine, serine, and tyrosine (SSY) at positions 100, 100a, and 100b of VH, which had 14-fold improved binding affinity owing to a lower off rate.[70] The resulting disulfide-stabilized recombinant immunotoxin, originally called HA22 for "higher affinity," was renamed CAT-8015 and, more recently, moxetumomab pasudotox (Figure 1). Phase 1 testing in 28 patients with HCL included 16 patients who received 5 to 40 mg/kg QOD × 3 and 12 patients who received 50 mg/kg QOD × 3. The CR rate was 46%, and the ORR was 86%.[39] Patients could receive up to 2 consolidation cycles (after documentation of CR), but treatment was stopped if significant levels of anti-drug antibodies or progressive disease was documented. A total of 114 cycles were administered to 28 patients. CR occurred after 2 to 5 (median, 3) cycles, with a total of 10 consolidation cycles given to 5 patients. CR was unrelated to the number of cycles of prior purine analogue or to the duration of response to the most recent purine analogue course. However, there were no CRs among 7 patients with prior splenectomy vs 13 CRs in 21 patients (62%) with a spleen (P=.007). Of the 13 CRs, 80% persisted for a median of 29 months. Neutralizing antibodies were documented in 10 of 26 evaluable patients (38%), but in only 1 patient after cycle 1, so that nearly all patients could be re-treated. No dose-limiting toxicity was observed, although 2 patients (8%) had grade 2 hemolytic uremic syndrome consisting of grade 1 (mild) changes in the platelet and creatinine levels, which rapidly recovered. The most common toxicities were related to mild capillary leak syndrome, possibly a result of immunotoxin passing through the endothelial cells lining the blood vessels; these included hypoalbuminemia, edema, hypotension, fatigue, weight gain, and proteinuria, usually grade 1. The trial continued with an expanded cohort at the highest dose, and patients with HCL treated on a pivotal phase 3 trial are currently under evaluation. Additional preclinical development is continuing as mutants of moxetumomab pasudotox with reduced immunogenicity are investigated.[71]

BRAF Targeting in Hairy Cell Leukemia

Both clinical and laboratory research on HCL were revolutionized when Italian investigators discovered that the *BRAF* V600E mutation, which is present in up to 50% of patients with malignant melanoma, is also present in nearly all patients with classic HCL.[16] This mutation causes constitutive activation of the MAP kinase pathway.[72,73] The *BRAF* V600E mutation is also seen not only in mature HCL cells but also in malignant hematopoietic stem cells.[74] The *BRAF* V600E mutation is found in only a few other hematologic disorders, including Langerhans cell histiocytosis,[75] multiple myeloma,[76] Erdheim-Chester disease,[77] and acute myelogenous leukemia.[78] Some patients with classic HCL have wild-type *BRAF*,[72,79] as do essentially all patients with HCLv and/or IGHV4-34 immunoglobulin rearrangement.[18] Up to 50% of these patients have mutations in *MAP2K1* encoding mitogen-activated protein kinase enzyme 1 (MEK1), including mutations in *U2AF1*, *ARID1A*, *TP53*, *TTN*, *KMT2C* (previously *MLL3*), and *CCND3*.[80,81]

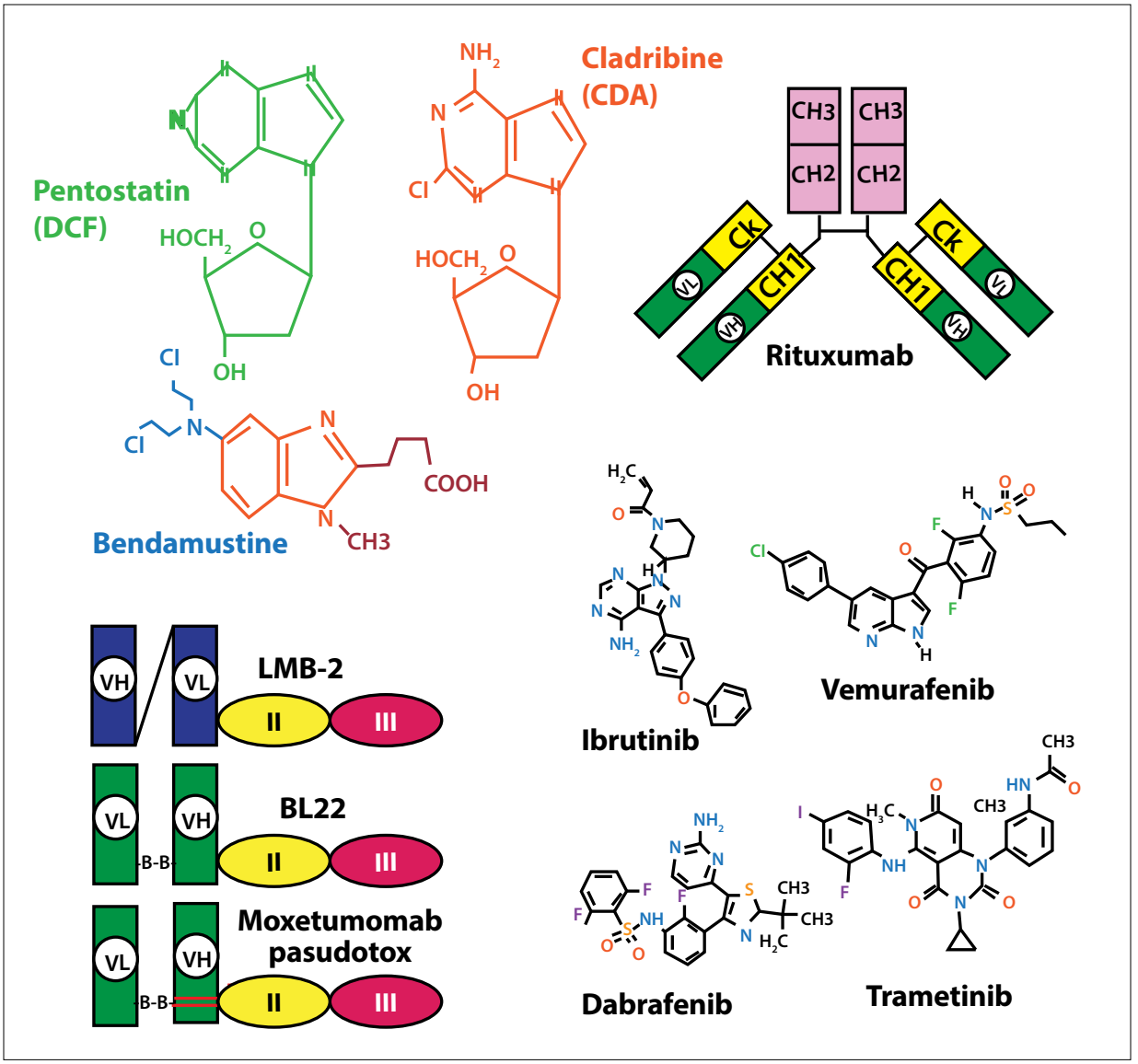

Figure 1. The schematic structures of anti–hairy cell leukemia agents. The traditional purine analogues pentostatin and cladribine have similar structures but slightly different mechanisms of action. Bendamustine contains the benzimidazole ring of cladribine, which makes it a purine analogue, but has alkylating groups for additional mechanisms of action. LMB-2 contains the variable domains (VH and VL) of the anti-CD25 monoclonal antibody anti-Tac connected by a $(G_4S)_3$ peptide linker, with VL fused to PE38. The anti-CD22 recombinant immunotoxins BL22 and moxetumomab pasudotox contain a disulfide-stabilized Fv made by engineering a disulfide bond into the framework regions, replacing Arg44 of VH and Gly100 of VL. In moxetumomab pasudotox, the amino acids 100, 100a, and 100b of VH, in the third complementarity-determining region (CDR3), are mutated from SSY (serine, serine, and tyrosine) to THW (threonine, histidine, and tryptophan), resulting in 14-fold improvement in binding affinity. Rituximab is a chimeric monoclonal antibody containing human CH3 and CH4 constant domains, whereas the remainder is of murine origin. The structures of the oral small molecules ibrutinib, vemurafenib, dabrafenib, and trametinib are shown.

CDA, 2-chlorodeoxyadenosine; DCF, 2-deoxycoformycin; Fv, variable fragment.

Clinical Development of BRAF Inhibition in Hairy Cell Leukemia

The kinase inhibitor vemurafenib (Zelboraf, Genentech/Daiichi Sankyo), approved in 2011 for *BRAF* V600E-positive melanoma, was reported effective in a patient with HCL.[82] Two HCL clinical trials, one in Italy and one in the United States, showed CR rates of 39% to 42% and ORRs of 96% to 100%.[83] Toxicities included rash and photosensitivity, arthralgias or arthritis, pyrexia, elevated bilirubin, and other laboratory abnormalities. Basal cell

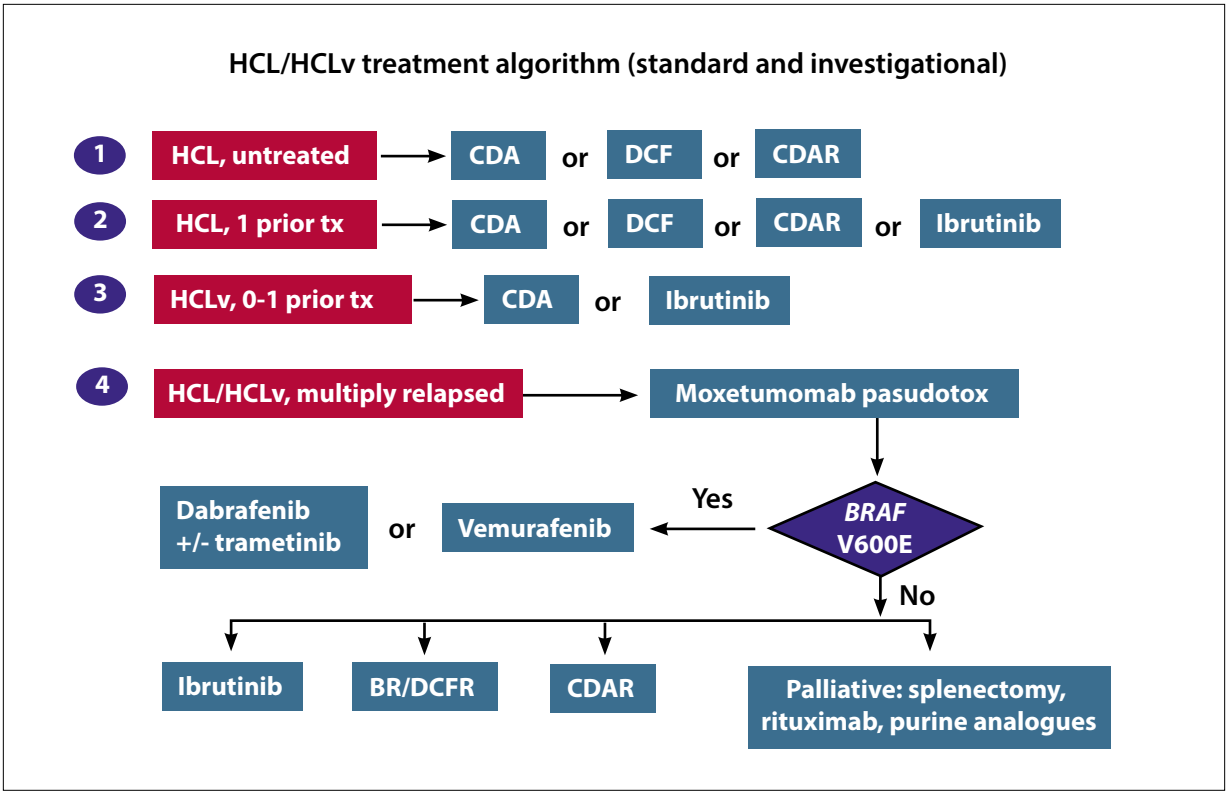

Figure 2. Treatment algorithm for HCL and HCLv. Both standard and investigational treatment options are shown for (1) patients with HCL without prior purine analogue therapy, (2) patients with HCL treated with 1 prior purine analogue, (3) patients with HCLv treated with 0 or 1 prior purine analogue, and (4) patients with multiply relapsed HCL/HCLv.

BR, bendamustine/rituximab; CDA, cladribine (2-chlorodeoxyadenosine); CDAR, cladribine/rituximab; DCF, pentostatin (2'-deoxycoformycin); DCFR, pentostatin/rituximab; HCL, hairy cell leukemia; HCLv, hairy cell leukemia variant; tx, therapy.

carcinomas requiring excision occurred in 2 patients. Although the responses occurred quickly, the Italian trial reported a median relapse-free survival of 9 months in responders and a median of 19 months for those with a CR. All patients with a CR in this group had evidence of MRD by IHC.[83] A retrospective study of low-dose vemurafenib (240 mg/day) was undertaken in HCL, and cytopenias improved in all of 21 patients.[84] Vemurafenib at a dose of 480 mg/day completely inhibited extracellular signal-regulated kinase phosphorylation. CRs were achieved in 6 of 15 evaluable patients (40%), but toxicities were not prevented by using low doses.[84] This study also reported that ERK is paradoxically activated in wild-type *BRAF* cells.[84]

To elucidate mutations that might interact with *BRAF* V600E, whole-exome and deep sequencing was done, showing mutations in *EZH2*, *ARID1A*, and cell cycle inhibitor *CDKN1B* (p27) as well as loss of chromosome 7q leading to loss of heterozygosity.[85] Mutations in *CDKN1B* or *KLF2*, reported in 16% of patients with HCL, are thought to interact with *BRAF* V600E to promote leukemic transformation.[72,73] *KLF2* mutations are usually loss-of-function mutations, and *CDKN1B* (p27) is a known tumor suppressor gene. Whole-exome sequencing of 5 patients with classic *BRAF* V600E-positive HCL showed a variety of nonrecurring somatic mutations.[86] Mutations reported in vemurafenib-resistant HCL include *IRS1*, *NF1*, and *NF2*.[80] *BRAF* status can be determined by using marrow IHC[87] and allele-specific or droplet PCR.[88] An international multicenter trial of the BRAF and MEK inhibitors dabrafenib (Tafinlar, Novartis) and trametinib (Mekinist, Novartis), also approved for *BRAF* V600E-positive melanoma, is currently underway in HCL and other diseases with *BRAF* V600E-positive histology. Dabrafenib alone achieved a CR in a patient with both HCL and melanoma.[89]

Bruton Tyrosine Kinase Inhibition in Hairy Cell Leukemia

The Bruton tyrosine kinase (BTK) inhibitor ibrutinib (Imbruvica, Pharmacyclics/Janssen) is approved for

chronic lymphocytic leukemia and mantle cell lymphoma. Ibrutinib affects the microenvironment, including matrix proteins, cytokines, and immune or accessory cells. The microenvironment, including CXCR4 and B-cell receptor–interacting proteins such as BTK, was shown to be important for the survival and expansion of HCL.[90] Uniform BTK expression is present in HCL cells, and ibrutinib can inhibit HCL proliferation and cell cycle progression.[91] A multicenter trial of ibrutinib in relapsed HCL and in HCLv is currently underway, coordinated by Ohio State University through the Center for Transdisciplinary Evidence-Based Practice. Excellent responses from this trial have been presented. In a recent case report, a patient who had multiply relapsed HCLv treated with ibrutinib derived clinical benefit with ibrutinib,[92] although the cytopenias did not resolve sufficiently for the benefit to be considered a PR.

Summary: Decisions About Therapy

The recent availability of approved and investigational options for HCL and HCLv has made it challenging to decide on therapy. Because few well-controlled clinical trials in HCL/HCLv have been published, consensus guidelines[33] are based more on clinical judgment than on scientific data. On the basis of both published trials and our experience in the treatment of HCL/HCLv with the investigational options discussed in this review, we propose the treatment algorithm that appears in Figure 2.

It is important to emphasize that patients should not start treatment until it is indicated. Importantly, patient harm can result when cytopenias that are due to recent therapy rather than persistent/recurrent HCL are treated. For patients with newly diagnosed classic HCL, treatment with a purine analogue such as cladribine or pentostatin is standard; the former is advantageous for completing therapy within 5 to 7 days, and the latter advantageous if more gradual treatment with reduction of the initial dose is preferred. To eliminate MRD early, an investigational first-line alternative is cladribine plus either delayed or concurrent rituximab.

Patients with once-relapsed HCL are often treated with a second-line purine analogue, but because inferior efficacy and cumulative toxicity may result, an alternative approach includes a purine analogue with rituximab. Patients with once-relapsed HCL are also eligible for the ibrutinib protocol, which is still accruing.

Our preference for multiply relapsed HCL is moxetumomab pasudotox, owing to its lack of chemotherapy-type toxicities and its ability to achieve CR and eliminate MRD long term. Our trial of rituximab with either pentostatin or bendamustine also efficiently eliminates MRD, but with chemotherapy toxicities. Oral nonchemotherapy options for multiply relapsed HCL include BRAF inhibition (either vemurafenib or dabrafenib/trametinib) or ibrutinib.

HCLv should not be treated with a single-agent purine analogue, even as first-line therapy. Cladribine plus rituximab is effective and well tolerated as either first- or second-line treatment for HCLv, and a study of ibrutinib is currently accruing patients with HCLv. Patients with multiply relapsed HCLv are appropriate candidates for moxetumomab pasudotox because HCLv retains a high level of expression of CD22, and rituximab with bendamustine or pentostatin is also appropriate for HCLv.

Additional options, or new combinations of existing options, will be welcome for patients with relapsed or refractory HCL or HCLv.

Acknowledgments

Research in this review was supported in part by the intramural program of the National Institutes of Health and the Hairy Cell Leukemia Foundation.

Disclosures

Dr Kreitman is a co-inventor on the patent for moxetumomab pasudotox, which has been assigned to the National Institutes of Health. Dr Arons has no disclosures to report.

References

1. Bouroncle BA, Wiseman BK, Doan CA. Leukemic reticuloendotheliosis. *Blood*. 1958;13(7):609-630.
2. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. *CA Cancer J Clin*. 2017;67(1):7-30.
3. Andritsos LA, Grever MR. Historical overview of hairy cell leukemia. *Best Pract Res Clin Haematol*. 2015;28(4):166-174.
4. Golomb HM, Catovsky D, Golde DW. Hairy cell leukemia: a clinical review based on 71 cases. *Ann Intern Med*. 1978;89(5 pt 1):677-683.
5. Habermann TM, Rai K. Historical treatments of in hairy cell leukemia, splenectomy and interferon: past and current uses. *Leuk Lymphoma*. 2011;52(suppl 2):18-20.
6. Bohn JP, Gastl G, Steurer M. Long-term treatment of hairy cell leukemia with interferon-α: still a viable therapeutic option. *Memo*. 2016;9:63-65.
7. Johnston JB, Glazer RI, Pugh L, Israels LG. The treatment of hairy-cell leukaemia with 2′-deoxycoformycin. *Br J Haematol*. 1986;63(3):525-534.
8. Spiers AS, Moore D, Cassileth PA, et al. Remissions in hairy-cell leukemia with pentostatin (2'-deoxycoformycin). *N Engl J Med*. 1987;316(14):825-830.
9. Piro LD, Carrera CJ, Carson DA, Beutler E. Lasting remissions in hairy-cell leukemia induced by a single infusion of 2-chlorodeoxyadenosine. *N Engl J Med*. 1990;322(16):1117-1121.
10. Cawley JC, Burns GF, Hayhoe FG. A chronic lymphoproliferative disorder with distinctive features: a distinct variant of hairy-cell leukaemia. *Leuk Res*. 1980;4(6):547-559.
11. Matutes E, Martínez-Trillos A, Campo E. Hairy cell leukaemia-variant: disease features and treatment. *Best Pract Res Clin Haematol*. 2015;28(4):253-263.
12. Tadmor T, Polliack A. Epidemiology and environmental risk in hairy cell leukemia. *Best Pract Res Clin Haematol*. 2015;28(4):175-179.
13. Arons E, Adams S, Venzon DJ, Pastan I, Kreitman RJ. Class II human leucocyte antigen DRB1*11 in hairy cell leukaemia patients with and without haemolytic uraemic syndrome. *Br J Haematol*. 2014;166(5):729-738.
14. Quest GR, Johnston JB. Clinical features and diagnosis of hairy cell leukemia. *Best Pract Res Clin Haematol*. 2015;28(4):180-192.
15. Shao H, Calvo KR, Grönborg M, et al. Distinguishing hairy cell leukemia

variant from hairy cell leukemia: development and validation of diagnostic criteria. *Leuk Res*. 2013;37(4):401-409.
16. Tiacci E, Trifonov V, Schiavoni G, et al. BRAF mutations in hairy-cell leukemia. *N Engl J Med*. 2011;364(24):2305-2315.
17. Wotherspoon A, Attygalle A, Mendes LS. Bone marrow and splenic histology in hairy cell leukaemia. *Best Pract Res Clin Haematol*. 2015;28(4):200-207.
18. Xi L, Arons E, Navarro W, et al. Both variant and IGHV4-34-expressing hairy cell leukemia lack the BRAF V600E mutation. *Blood*. 2012;119(14):3330-3332.
19. Ravandi F, O'Brien S, Jorgensen J, et al. Phase 2 study of cladribine followed by rituximab in patients with hairy cell leukemia. *Blood*. 2011;118(14):3818-3823.
20. Kreitman RJ, Wilson W, Calvo KR, et al. Cladribine with immediate rituximab for the treatment of patients with variant hairy cell leukemia. *Clin Cancer Res*. 2013;19(24):6873-6881.
21. Matutes E, Oscier D, Montalban C, et al. Splenic marginal zone lymphoma proposals for a revision of diagnostic, staging and therapeutic criteria. *Leukemia*. 2008;22(3):487-495.
22. Arons E, Suntum T, Stetler-Stevenson M, Kreitman RJ. VH4-34+ hairy cell leukemia, a new variant with poor prognosis despite standard therapy. *Blood*. 2009;114(21):4687-4695.
23. Jain P, Ok CY, Konoplev S, et al. Relapsed refractory BRAF-negative, IGHV4-34-positive variant of hairy cell leukemia: a distinct entity? *J Clin Oncol*. 2016;34(7):e57-e60.
24. Arons E, Kreitman RJ. Molecular variant of hairy cell leukemia with poor prognosis. *Leuk Lymphoma*. 2011;52(suppl 2):99-102.
25. Poret N, Fu Q, Guihard S, et al. CD38 in hairy cell leukemia is a marker of poor prognosis and a new target for therapy. *Cancer Res*. 2015;75(18):3902-3911.
26. Jain D, Dorwal P, Gajendra S, Pande A, Mehra S, Sachdev R. CD5 positive hairy cell leukemia: A rare case report with brief review of literature. *Cytometry B Clin Cytom*. 2016;90(5):467-472.
27. Shackelford RE, Heldmann M, Eskandari F, et al. Marked retroperitoneal lymphadenopathy in hairy cell leukemia: a case report. *Case Rep Oncol*. 2013;6(3):493-496.
28. Cortazar JM, DeAngelo DJ, Pinkus GS, Morgan EA. Morphological and immunophenotypical features of hairy cell leukaemia involving lymph nodes and extranodal tissues. *Histopathology*. 2017;71(1):112-124.
29. Wang L, Tadros AS, Hoh CK, Wang HY. CD10-positive hairy cell leukemia involving multiple deep lymph nodes. *Clin Lymphoma Myeloma Leuk*. 2016;16(5):e51-e53.
30. Tabata R, Tabata C, Iwama H, Yasumizu R, Kojima M. CD27-positive hairy cell leukemia-Japanese variant. *Virchows Arch*. 2016;468(3):375-379.
31. Grever M, Kopecky K, Foucar MK, et al. Randomized comparison of pentostatin versus interferon alfa-2a in previously untreated patients with hairy cell leukemia: an intergroup study. *J Clin Oncol*. 1995;13(4):974-982.
32. Saven A, Burian C, Koziol JA, Piro LD. Long-term follow-up of patients with hairy cell leukemia after cladribine treatment. *Blood*. 1998;92(6):1918-1926.
33. Grever MR, Abdel-Wahab O, Andritsos LA, et al. Consensus guidelines for the diagnosis and management of patients with classic hairy cell leukemia. *Blood*. 2017;129(5):553-560.
34. Kreitman RJ, Wilson WH, Robbins D, et al. Responses in refractory hairy cell leukemia to a recombinant immunotoxin. *Blood*. 1999;94(10):3340-3348.
35. Kreitman RJ, Wilson WH, White JD, et al. Phase I trial of recombinant immunotoxin anti-Tac(Fv)-PE38 (LMB-2) in patients with hematologic malignancies. *J Clin Oncol*. 2000;18(8):1622-1636.
36. Kreitman RJ, Wilson WH, Bergeron K, et al. Efficacy of the anti-CD22 recombinant immunotoxin BL22 in chemotherapy-resistant hairy-cell leukemia. *N Engl J Med*. 2001;345(4):241-247.
37. Kreitman RJ, Squires DR, Stetler-Stevenson M, et al. Phase I trial of recombinant immunotoxin RFB4(dsFv)-PE38 (BL22) in patients with B-cell malignancies. *J Clin Oncol*. 2005;23(27):6719-6729.
38. Kreitman RJ, Stetler-Stevenson M, Margulies I, et al. Phase II trial of recombinant immunotoxin RFB4(dsFv)-PE38 (BL22) in patients with hairy cell leukemia. *J Clin Oncol*. 2009;27(18):2983-2990.
39. Kreitman RJ, Tallman MS, Robak T, et al. Phase I trial of anti-CD22 recombinant immunotoxin moxetumomab pasudotox (CAT-8015 or HA22) in patients with hairy cell leukemia. *J Clin Oncol*. 2012;30(15):1822-1828.
40. Else M, Dearden CE, Matutes E, et al. Long-term follow-up of 233 patients with hairy cell leukaemia, treated initially with pentostatin or cladribine, at a median of 16 years from diagnosis. *Br J Haematol*. 2009;145(6):733-740.
41. Flinn IW, Kopecky KJ, Foucar MK, et al. Long-term follow-up of remission duration, mortality, and second malignancies in hairy cell leukemia patients treated with pentostatin. *Blood*. 2000;96(9):2981-2986.
42. Seymour JF, Kurzrock R, Freireich EJ, Estey EH. 2-chlorodeoxyadenosine induces durable remissions and prolonged suppression of CD4+ lymphocyte counts in patients with hairy cell leukemia. *Blood*. 1994;83(10):2906-2911.
43. Seymour JF, Talpaz M, Kurzrock R. Response duration and recovery of CD4+ lymphocytes following deoxycoformycin in interferon-alpha-resistant hairy cell leukemia: 7-year follow-up. *Leukemia*. 1997;11(1):42-47.
44. Cheson BD, Sorensen JM, Vena DA, et al. Treatment of hairy cell leukemia with 2-chlorodeoxyadenosine via the Group C protocol mechanism of the National Cancer Institute: a report of 979 patients. *J Clin Oncol*. 1998;16(9):3007-3015.
45. Else M, Dearden CE, Catovsky D. Long-term follow-up after purine analogue therapy in hairy cell leukaemia. *Best Pract Res Clin Haematol*. 2015;28(4):217-229.
46. Rosenberg JD, Burian C, Waalen J, Saven A. Clinical characteristics and long-term outcome of young hairy cell leukemia patients treated with cladribine: a single-institution series. *Blood*. 2014;123(2):177-183.
47. Getta BM, Woo KM, Devlin S, et al. Treatment outcomes and secondary cancer incidence in young patients with hairy cell leukaemia. *Br J Haematol*. 2016;175(3):402-409.
48. Consensus resolution: proposed criteria for evaluation of response to treatment in hairy cell leukemia. *Leukemia*. 1987;1(4):405.
49. Chadha P, Rademaker AW, Mendiratta P, et al. Treatment of hairy cell leukemia with 2-chlorodeoxyadenosine (2-CdA): long-term follow-up of the Northwestern University experience. *Blood*. 2005;106(1):241-246.
50. Burotto M, Stetler-Stevenson M, Arons E, Zhou H, Wilson W, Kreitman RJ. Bendamustine and rituximab in relapsed and refractory hairy cell leukemia. *Clin Cancer Res*. 2013;19(22):6313-6321.
51. Tallman MS, Hakimian D, Kopecky KJ, et al. Minimal residual disease in patients with hairy cell leukemia in complete remission treated with 2-chlorodeoxyadenosine or 2-deoxycoformycin and prediction of early relapse. *Clin Cancer Res*. 1999;5(7):1665-1670.
52. Arons E, Margulies I, Sorbara L, et al. Minimal residual disease in hairy cell leukemia patients assessed by clone-specific polymerase chain reaction. *Clin Cancer Res*. 2006;12(9):2804-2811.
53. Villamor N, Montserrat E, Colomer D. Mechanism of action and resistance to monoclonal antibody therapy. *Semin Oncol*. 2003;30(4):424-433.
54. Nieva J, Bethel K, Saven A. Phase 2 study of rituximab in the treatment of cladribine-failed patients with hairy cell leukemia. *Blood*. 2003;102(3):810-813.
55. Ravandi F. Chemoimmunotherapy for hairy cell leukemia. *Best Pract Res Clin Haematol*. 2015;28(4):230-235.
56. Chow KU, Sommerlad WD, Boehrer S, et al. Anti-CD20 antibody (IDEC-C2B8, rituximab) enhances efficacy of cytotoxic drugs on neoplastic lymphocytes in vitro: role of cytokines, complement, and caspases. *Haematologica*. 2002;87(1):33-43.
57. Chaudhary VK, Queen C, Junghans RP, Waldmann TA, FitzGerald DJ, Pastan I. A recombinant immunotoxin consisting of two antibody variable domains fused to *Pseudomonas* exotoxin. *Nature*. 1989;339(6223):394-397.
58. Kreitman RJ, Pastan I. Targeting *Pseudomonas* exotoxin to hematologic malignancies. *Semin Cancer Biol*. 1995;6(5):297-306.
59. Kreitman RJ, Pastan I. Accumulation of a recombinant immunotoxin in a tumor in vivo: fewer than 1000 molecules per cell are sufficient for complete responses. *Cancer Res*. 1998;58(5):968-975.
60. Kreitman RJ, Chaudhary VK, Kozak RW, FitzGerald DJP, Waldman TA, Pastan I. Recombinant toxins containing the variable domains of the anti-Tac monoclonal antibody to the interleukin-2 receptor kill malignant cells from patients with chronic lymphocytic leukemia. *Blood*. 1992;80(9):2344-2352.
61. Kreitman RJ, Stetler-Stevenson M, Jaffe ES, et al. Complete remissions of adult T-cell leukemia with anti-CD25 recombinant immunotoxin LMB-2 and chemotherapy to block immunogenicity. *Clin Cancer Res*. 2016;22(2):310-318.
62. Robbins DH, Margulies I, Stetler-Stevenson M, Kreitman RJ. Hairy cell leukemia, a B-cell neoplasm that is particularly sensitive to the cytotoxic effect of anti-Tac(Fv)-PE38 (LMB-2). *Clin Cancer Res*. 2000;6(2):693-700.
63. Amlot PL, Stone MJ, Cunningham D, et al. A phase I study of an anti-CD22-deglycosylated ricin A chain immunotoxin in the treatment of B-cell lymphomas resistant to conventional therapy. *Blood*. 1993;82(9):2624-2633.
64. Kreitman RJ, Hansen HJ, Jones AL, FitzGerald DJP, Goldenberg DM, Pastan I. *Pseudomonas* exotoxin-based immunotoxins containing the antibody LL2 or LL2-Fab' induce regression of subcutaneous human B-cell lymphoma in mice. *Cancer Res*. 1993;53(4):819-825.
65. Mansfield E, Pastan I, FitzGerald DJ. Characterization of RFB4-Pseudomonas exotoxin A immunotoxins targeted to CD22 on B-cell malignancies. *Bioconjug Chem*. 1996;7(5):557-563.
66. Mansfield E, Amlot P, Pastan I, FitzGerald DJ. Recombinant RFB4 immuno-

toxins exhibit potent cytotoxic activity for CD22-bearing cells and tumors. *Blood*. 1997;90(5):2020-2026.
67. Reiter Y, Brinkmann U, Kreitman RJ, Jung S-H, Lee B, Pastan I. Stabilization of the Fv fragments in recombinant immunotoxins by disulfide bonds engineered into conserved framework regions. *Biochemistry*. 1994;33(18):5451-5459.
68. Kreitman RJ, Margulies I, Stetler-Stevenson M, Wang QC, FitzGerald DJP, Pastan I. Cytotoxic activity of disulfide-stabilized recombinant immunotoxin RFB4(dsFv)-PE38 (BL22) toward fresh malignant cells from patients with B-cell leukemias. *Clin Cancer Res*. 2000;6(4):1476-1487.
69. Kreitman RJ, Wang QC, FitzGerald DJP, Pastan I. Complete regression of human B-cell lymphoma xenografts in mice treated with recombinant anti-CD22 immunotoxin RFB4(dsFv)-PE38 at doses tolerated by cynomolgus monkeys. *Int J Cancer*. 1999;81(1):148-155.
70. Salvatore G, Beers R, Margulies I, Kreitman RJ, Pastan I. Improved cytotoxic activity toward cell lines and fresh leukemia cells of a mutant anti-CD22 immunotoxin obtained by antibody phage display. *Clin Cancer Res*. 2002;8(4):995-1002.
71. Mazor R, Eberle JA, Hu X, et al. Recombinant immunotoxin for cancer treatment with low immunogenicity by identification and silencing of human T-cell epitopes. *Proc Natl Acad Sci U S A*. 2014;111(23):8571-8576.
72. Falini B, Martelli MP, Tiacci E. BRAF V600E mutation in hairy cell leukemia: from bench to bedside. *Blood*. 2016;128(15):1918-1927.
73. Tiacci E, Pettirossi V, Schiavoni G, Falini B. Genomics of hairy cell leukemia. *J Clin Oncol*. 2017;35(9):1002-1010.
74. Chung SS, Kim E, Park JH, et al. Hematopoietic stem cell origin of BRAFV600E mutations in hairy cell leukemia. *Sci Transl Med*. 2014;6(238):238ra71.
75. Kobayashi M, Tojo A. The BRAF-V600E mutation in circulating cell-free DNA is a promising biomarker of high-risk adult Langerhans cell histiocytosis. *Blood*. 2014;124(16):2610-2611.
76. Walker BA, Boyle EM, Wardell CP, et al. Mutational spectrum, copy number changes, and outcome: results of a sequencing study of patients with newly diagnosed myeloma. *J Clin Oncol*. 2015;33(33):3911-3920.
77. Campochiaro C, Dagna L. BRAF Mutations in Erdheim-Chester disease. *Dermatol Clin*. 2016;34(1):xi-xii.
78. Smith ML, Snaddon J, Neat M, et al. Mutation of BRAF is uncommon in AML FAB type M1 and M2. *Leukemia*. 2003;17(1):274-275.
79. Kreitman RJ. Hairy cell leukemia-new genes, new targets. *Curr Hematol Malig Rep*. 2013;8(3):184-195.
80. Durham BH, Getta B, Dietrich S, et al. Genomic analysis of hairy cell leukemia identifies novel recurrent genetic alterations. *Blood*. 2017;130(14):1644-1648.
81. Waterfall JJ, Arons E, Walker RL, et al. High prevalence of MAP2K1 mutations in variant and IGHV4-34-expressing hairy-cell leukemias. *Nat Genet*. 2014;46(1):8-10.
82. Dietrich S, Glimm H, Andrulis M, von Kalle C, Ho AD, Zenz T. BRAF inhibition in refractory hairy-cell leukemia. *N Engl J Med*. 2012;366(21):2038-2040.
83. Tiacci E, Park JH, De Carolis L, et al. Targeting mutant BRAF in relapsed or refractory hairy-cell leukemia. *N Engl J Med*. 2015;373(18):1733-1747.
84. Dietrich S, Pircher A, Endris V, et al. BRAF inhibition in hairy cell leukemia with low-dose vemurafenib. *Blood*. 2016;127(23):2847-2855.
85. Dietrich S, Hüllein J, Lee SC, et al. Recurrent CDKN1B (p27) mutations in hairy cell leukemia. *Blood*. 2015;126(8):1005-1008.
86. Weston-Bell NJ, Tapper W, Gibson J, et al. Exome sequencing in classic hairy cell leukaemia reveals widespread variation in acquired somatic mutations between individual tumours apart from the signature BRAF V(600)E lesion. *PLoS One*. 2016;11(2):e0149162.
87. Brown NA, Betz BL, Weigelin HC, Elenitoba-Johnson KS, Lim MS, Bailey NG. Evaluation of allele-specific PCR and immunohistochemistry for the detection of BRAF V600E mutations in hairy cell leukemia. *Am J Clin Pathol*. 2015;143(1):89-99.
88. Guerrini F, Paolicchi M, Ghio F, et al. The droplet digital PCR: a new valid molecular approach for the assessment of B-RAF V600E mutation in hairy cell leukemia. *Front Pharmacol*. 2016;7:363.
89. Blachly JS, Lozanski G, Lucas DM, Grever MR, Kendra K, Andritsos LA. Cotreatment of hairy cell leukemia and melanoma with the BRAF inhibitor dabrafenib. *J Natl Compr Canc Netw*. 2015;13(1):9-13.
90. Burger JA, Sivina M, Ravandi F. The microenvironment in hairy cell leukemia: pathways and potential therapeutic targets. *Leuk Lymphoma*. 2011;52(suppl 2):94-98.
91. Sivina M, Kreitman RJ, Arons E, Ravandi F, Burger JA. The bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) blocks hairy cell leukaemia survival, proliferation and B cell receptor signalling: a new therapeutic approach. *Br J Haematol*. 2014;166(2):177-188.
92. Bohn JP, Wanner D, Steurer M. Ibrutinib for relapsed refractory hairy cell leukemia variant. *Leuk Lymphoma*. 2017;58(5):1224-1226.