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Leukemia (PH Wiernik, Section Editor)

Biology and Treatment of Hairy Cell Leukemia

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Opinion statement

Despite its rarity, hairy cell leukemia (HCL) remains a fascinating disease and the physiopathology is becoming more and more understood. The accurate diagnosis of HCL relies on the recognition of hairy cells by morphology and flow cytometry (FCM) in the blood and/or bone marrow (BM). The *BRAF* V600E mutation, an HCL-defining mutation, represents a novel diagnostic parameter and a potential therapeutic target. The precise cellular origin of HCL is a late-activated postgerminal center memory B cell. BRAF mutations were detected in hematopoietic stem cells (HSCs) of patients with HCL, suggesting that this is an early HCL-defining event. Watch-and-wait strategy is necessary in approximately 10% of asymptomatic HCL patients, sometimes for several years. Purine analogs (PNAs) are the established first-line options for symptomatic HCL patients. In second-line treatment, chemoimmunotherapy combining PNA plus rituximab should be considered in high-risk HCL patients. The three options for relapsed/refractory HCL patients include recombinant immunoconjugates targeting CD22, BRAF inhibitors, and BCR inhibitors. The clinical interest to investigate blood minimal residual disease (MRD) was recently demonstrated, with a high risk of relapse in patients with positive testing for MRD and a low risk in patients with negative testing. However, efforts must be made to standardize MRD analyses in the near future. Patients with HCL are at risk of second malignancies. The increased risk could be related to the disease and/or the treatment, and the respective role of PNAs in the development of secondary malignancies remains a topic of debate.

Introduction

tpdelairy cell leukemia (HCL) is an uncommon chronic leukemia of abnormal, clonal, mature B cells. Recognized as an entity by the World Health Organization in 2016 [1], HCL accounts for 2% of all leukemias, with approximately 1240 new HCL cases expected per year in the USA [2] and a relatively stable overall age-standardized incidence ratio [3–6] of 0.5/100000 person-years for men and 0.1/100000 for women. The median age at diagnosis is 63 years for men and 59 years for women [7]. A significantly lower overall survival (OS) is observed among African American individuals than among other ethnic groups [6]. Familial HCL is rare with fewer than 20 families reported in the literature [8]: an HLA-linked disorder was suggested [9] and no genetic susceptibility variants have been identified. Relatives of chronic lymphocytic leukemia (CLL) patients are at risk for developing CLL (RR =8.5, 6.1–11.7) and other non-Hodgkin's lymphomas (NHLs) (RR =1.9, 1.5–2.3), particularly lymphoplasmacytic lymphoma/Waldenström macroglobulinemia and HCL [10]. HCL must be differentiated from other HCL-like disorders, including hairy cell leukemia variant (HCL-V), representing approximately 10% of all HCL [11], splenic diffuse red pulp lymphoma (SDRPL) [12] and splenic marginal zone lymphoma (SMZL). The *BRAF* V600E mutation was observed in almost all HCL; conversely, it was not identified in HCL-V and SDRPL and was present at an exceptionally low rate in SMZL [13]. HCL was historically treated with splenectomy and then interferon-alpha (IFNα), and the prognosis of HCL changed with the use of purine analogs (PNAs), such as cladribine (2-CdA) or pentostatin (DCF), with a 10-year OS of 90%. In this article, we will review the significant advances over the last 3 years in the understanding of HCL and HCL-like disorders and provide an update on new treatment procedures that are now available, particularly for patients with relapsed/refractory HCL.

How has the diagnosis of HCL and HCL-like disorders improved in daily practice?

At diagnosis, HCL is usually characterized by infections, splenomegaly, or the presence of cytopenias. Autoimmune manifestations or unusual manifestations have also be identified, some of which can mimic multiple myeloma [14, 15]. With the high frequency of routine peripheral blood analyses, hairy cells can also be detected in asymptomatic patients. Complete blood counts and a careful review of peripheral blood smears are the first steps in the identification of cytologically characteristic hairy cells (which have irregular projections and clumps of microvilli on the surface of the cells) (Fig. 1). The hairy morphology could be due to Rho GTPases, such as RhoA, Rac1, and Cdc42, which are constitutively overexpressed in HCL. Beta-actin and leukocyte-specific transcript 1 (LST1) could be reorganized by these GTPases [16].

Using flow cytometry (FCM) to analyze the blood and/or marrow, high CD19, CD20, CD22, and CD200 [17] expression is usually observed. Hairy cells are negative for CD5, CD23, CD10, and CD27 but are positive for CD11c, CD25, CD103, and CD123. An immunological score was proposed [18•] in which one point was given for each of the last four markers when they were expressed and no points were given when they were not expressed. A score of 3 or 4 is observed in 98% of HCL patients, whereas in other HCL-like disorders, the score is usually low (0 or 1). CD38 is expressed in one-third of HCL and is a poor prognostic marker [19].

In the international consensus guidelines [20••], trephine bone marrow (BM) biopsy has been shown to reflect the degree of tumor infiltration and to

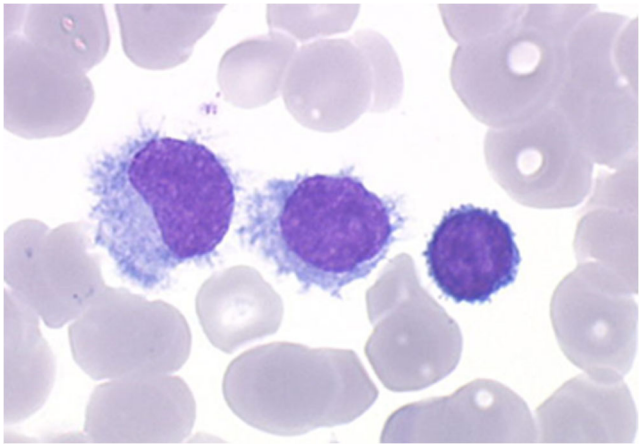

Fig. 1. Cytological aspects of hairy cell leukemia (HCL). Courtesy to Dr. Franck Genevieve, Laboratory of Hematology, Academic Hospital of Angers, Angers, Pays de la Loire, France.

help to diagnose complex cases. Hairy cells are positive for tartrate-resistant acid phosphatase (TRAP), annexin A1, and cyclin D1.

HCL must be distinguished from HCL-V, SDRPL, and SMZL. There are probably overlaps between all of these entities. Identifying these diseases is a real challenge in real life because the therapeutic management of these distinct diseases is completely different. HCL-V is a provisional entity in which circulating abnormal lymphoid cells have a morphology that is intermediate between prolymphocytes and hairy cells. HCL-V cells do not express CD25 and have weak CD123 expression. In SDRPL, a homogeneous infiltration of a large proportion (median 60%) of small- to medium-sized villous lymphoid cells is usually observed in the peripheral blood. The abnormal lymphoid cells in SDRPL have a polar distribution of villi, and the nucleolus is small or not visible. Additionally, monoclonal B cells express CD11c (97%) and CD103 (38%) and rarely express CD123 (16%) or CD25 (3%) in SDRPL. A scoring system based on CD11c, CD22, CD76, CD38, and CD27 was designed to differentiate SDRPL from SMZL. In addition, the CD200/CD180 median fluorescence intensity (MFI) ratio may be helpful to distinguish HCL from SDRPL, with a ratio of 0.5 or less suggesting SDRPL [21, 22].

What has recently improved our understanding of HCL?

Major progress has been made in recent years regarding the understanding of disease pathobiology (Fig. 2).

BRAF V600E mutation: an HCL-defining event

The B-raf proto-oncogene (*BRAF* gene) (7q34) is composed of 18 exons. The *BRAF* V600E mutation occurs in exon 15 at position 1799, at which thymine and adenine are exchanged, leading to valine (V) being replaced by glutamate (E) at codon 600 (V600E) of the BRAF protein. The mutation is considered the HCL-defining mutation and represents a novel diagnostic possibility; additionally, this mutation suggests that using BRAF inhibitors (vemurafenib and

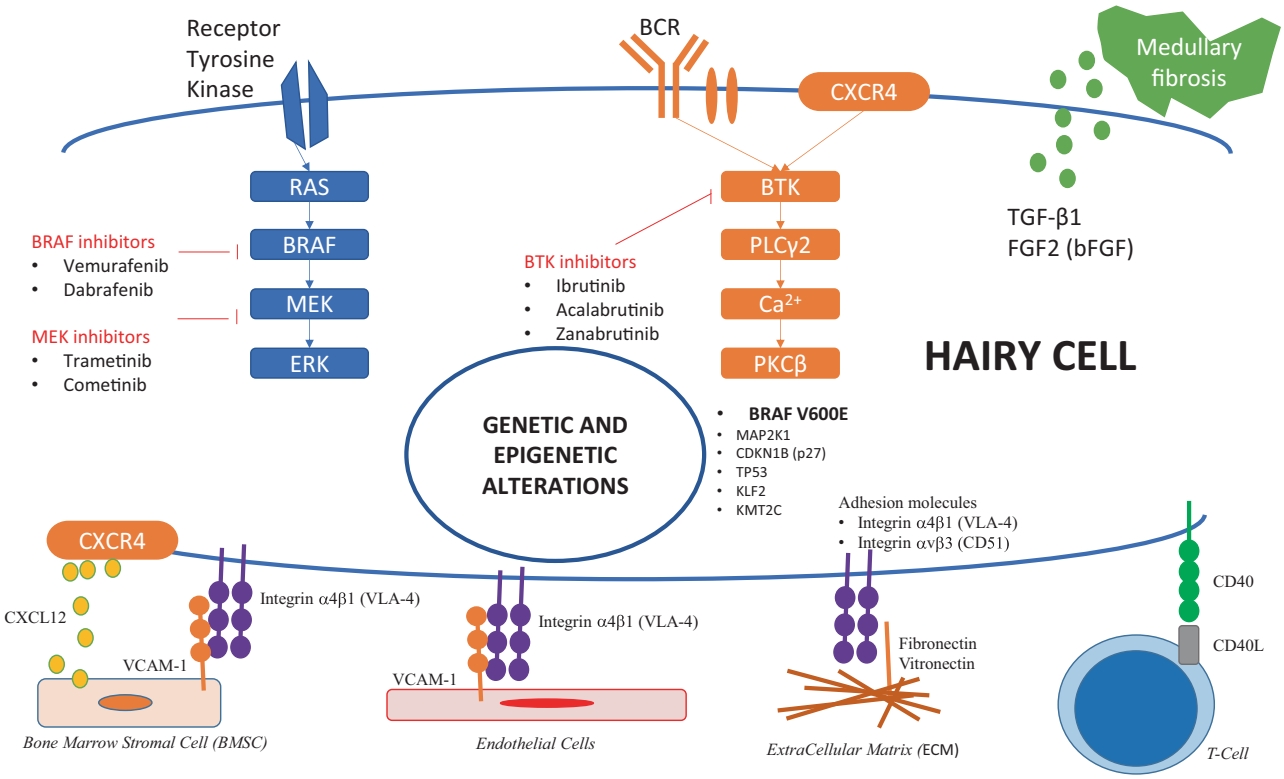

Fig. 2. Biology of hairy cell leukemia.

dabrafenib) may be an option (Fig. 2). In 2011, this mutation was identified in an HCL patient using whole-exome sequencing (WES) and was confirmed in 47 additional HCL patients using Sanger DNA sequencing. The mutation was not identified in 195 other patients with B cell chronic lymphoproliferative disorders, including 22 with SMZL and 16 with unclassifiable splenic B cell lymphomas who had either HCL-V or SDRPL [23••]. *BRAF* V600E-negativity was subsequently reported in up to 10% to 20% of HCL patients; in those patients, a mutation in exon 11 (F468C or D449E) should be excluded [24•]. Conversely, BRAF mutations in either exon 11 or 15 were not detected in 16 HCL-V patients, suggesting that HCL and HCL-V could represent different entities [25••, 26].

Other genes are recurrently mutated in HCL: role in disease progression

The sequencing of sequential samples at diagnosis and relapse demonstrated the emergence of new mutations at relapse [27••]. Alterations of the cell cycle are essential in the pathology of HCL. In addition to the overexpression of cyclin D1, the recurrent inactivation of the cell cycle inhibitor *CDKN1B/p27* was identified in more than 10% of patients [28]. Although *TP53* mutations and/or del(17p) seem to be infrequent [29••, 30–32] in HCL, it is recommended that the *TP53* status be assessed in cases of PNA resistance [33]. Inactivating *KLF2* mutations were observed in 15% of HCL cases [27••] and in 30% of marginal zone lymphomas (MZLs) [13]. KLF2 is a transcription factor that controls the differentiation of multiple B cell subpopulations, including marginal zone B

cells. Mutations in epigenetic regulatory genes were frequently observed, with mutations in the histone methyltransferase *KMT2C* (MLL3) occurring in 15% of patients [30]; more rarely, mutations in the histone demethylase *KDM6A* or the histone acetyltransferase *CREBBP* (*CBP*) were observed (Fig. 2) [27••, 30]. Other mutations in the chromatin remodeling complex family *ARID1A* and *ARID1B* [27••] have also been described.

IGHV4-34-positive and *BRAF* V600E-negative HCL: a new subgroup of HCL with a poor prognosis

The immunoglobulin heavy chain variable region gene *(IGHV)* is mutated in 90% of HCL patients. Patients with an unmutated status have a shorter OS than those with a mutated status. Forty percent of HCL-V and 10% of HCL patients have the *IGHV4-34* immunoglobulin variable heavy chain rearrangement. *VH4-34*-positive HCL patients represent a subgroup of patients with a higher disease burden at diagnosis, poor responses to PNA, shorter OS, and the absence of *BRAF* V600E mutations [29••, 34].

High prevalence of activating *MAP2K1* mutations in HCL-V and *IGHV4-34*-positive HCL

The WES of 10 HCL samples showed CD103+ and CD25- HCL-V in 5 patients, *VH4-34*+ hairy cell proliferation in 3 patients, both CD103+ and CD25- HCL-V and *VH4-34*+ hairy cell proliferation in 2 patients, and a high prevalence of activating mutations in the mitogen-activated protein kinase 1 (*MAP2K1)* gene (15q22.1-q22.3) in 5 of 10 samples. *MAP2K1* mutations were subsequently validated in a set of 21 additional patients. Sanger sequencing of exons 2 and 3 of *MAP2K1* identified ten additional positive samples, resulting in an overall frequency of 48%. Interestingly, *MAP2K1* mutations were identified in all three subgroups that were tested: 6/15 IGHV4-34-negative HCL-V patients, 4/9 IGHV4-34-positive HCL-V patients, and 5/7 IGHV4-34-positive HCL patients. In contrast, *MAP2K1* mutations were identified in only 1/20 IGHV4-34-negative HCL patients [25••].

High prevalence of *CCND3* and *U2AF1* mutations in HCL-V

In contrast to HCL patients, mutations in *CCND3* were observed in 13% of HCL-V patients [30]; an identical frequency was observed in SMZL patients, and *CCND3* mutations were observed in less than 25% of SDRPL patients [35]. *CCND3* mutations involve the regulatory PEST domain and lead to cyclin D3 overexpression. Recurrent hotspot mutations in *U2AF1*, which encodes a protein belonging to the spliceosome, were also detected in 15% of HCL-V [25••, 30].

SDRPL: an entity that is very similar to HCL-V

SDRPL was first described in 37 patients [12]. The homogeneous infiltration of a large proportion (median 60%) of small- to medium-sized villous lymphoid cells was usually observed in the peripheral blood. The abnormal lymphoid cells had a polar distribution of the villi, and the nucleolus was small or not visible. The abnormal B cells expressed CD11c (97%) and CD103 (38%) and rarely expressed CD123 (16%) or CD25 (3%). Most cases displayed a mutated *IGHV* status, with selective *IGHV* gene usage and an overrepresentation of *IGHV4-34*. The *BRAF* V600E mutation was never detected.

Cellular origin of HCL cells

Late-activated postgerminal center memory B cells were considered the normal counterpart of hairy cells [36]. The presence of mutated IGHV in 90% of HCL and a recent methylome analysis of several B cell neoplasms support this origin [37••]. Transforming events occur in the hematopoietic stem cell (HSC) compartment [38••], and HSCs contain the BRAF mutation. In addition, the expression of *BRAF* V600E in murine hematopoietic stem/progenitor cells can cause HCL-like disease. If memory B cells are the normal counterpart of hairy cells, transforming events occur at the HSC level.

Signaling pathways

Signaling pathways were implicated in the pathophysiology of HCL, offering new therapeutic targets in HCL (Fig. 2). The RAF-MEK-ERK pathway is constitutively activated [39, 40] as a result of the presence of the *BRAF* V600E mutation in most HCL patients and *MAP2K1* mutations in approximately 50% of HCL-V cases. The PI3Kinase-Akt pathway also plays an important role in the survival of HCL cells [40]. A role for the CD40, CXCR4, and B cell receptor (BCR) pathways was more recently demonstrated [41, 42]. The BCR pathway is constitutively activated in HCL, allowing the proliferation and survival of hairy cells. BCR crosslinking induces the phosphorylation of Bruton's tyrosine kinase (BTK) at Y223, the activation of MAPK and Akt, and the secretion of CCR3/CCR4. Ibrutinib, a BTK inhibitor, inhibits the proliferation and survival of HCL cells in vitro. The preclinical data support the potential usefulness of ibrutinib in HCL [42].

Tumor microenvironment

Several studies have shown that there are many interactions between hairy cells and other cells, such as stromal cells, endothelial cells, and immune cells, and between hairy cells and the extracellular matrix (ECM). Chemokine receptors (such as CXCR4) and adhesion molecules (such as VLA-4) play an important role in these interactions via cell-cell contact or paracrine interactions. These interactions have a protective effect on hairy cells: they inhibit apoptosis and promote proliferation and survival [43]. Moreover, the overexpression of TGF-β1 and FGF2 in hairy cells may explain the frequent BM fibrosis (Fig. 2) [40, 41].

Undetectable minimal residual disease (MRD): the first step to a cure

The clinical relevance of investigating blood MRD was demonstrated, as there is a high risk of relapse in patients with positive MRD test results and a low risk in patients with negative test results. FCM using an 8-color panel (CD103/CD305/CD19/CD123/CD25/CD3/CD45/CD20) was used. Only 1/9 patients who achieved a hematological response and MRD $<10^{-4}$ cells in at least two consecutive blood samples during the first 2 years after PNA treatment relapsed compared with 5/6 patients with MRD $>10^{-4}$ cells [44]. Validation based on the immunohistochemical diagnosis of HCL using a VE1 antibody specific to BRAF V600E-mutated cells was recently established and could represent a simple preliminary approach to detect MRD in clinical practice [45]. Other studies used polymerase chain reaction testing or immunohistochemistry (IHC) using PAX5/CD103 and PAX5/TRAP dual

IHC stains [46]. Efforts must be made to standardize MRD analyses in the near future.

Updates in treatment options for HCL

In the Surveillance Epidemiology and End Results (SEER) database analysis on survival in patients diagnosed with HCL in the USA between 1978 and 2008, an improvement in survival was observed over time (before 1984, 1984–1990, 1991–1999, and 2000–2008), with a reduction in the risk of mortality of 6.5% per year, clearly demonstrating a significant improvement in the clinical management of patients with HCL [47]. Varied treatment options are available (Fig. 3).

Watch-and-wait strategy

This strategy is necessary in approximately 10% of asymptomatic HCL patients, sometimes for several years.

PNAs: established first-line options for symptomatic HCL patients

Patients should be treated if they have symptoms from the disease or if their hematologic parameters are declining. The hematologic parameters that indicate that treatment is needed include at least one of the following: hemoglobin <11 g/dL; platelet count <100,000/μL; and absolute neutrophil count <1000/μL. Symptomatic splenomegaly may serve as an indication for treatment. Treatment is based on either 2-CdA or DCF. However, no randomized trials have compared DCF and 2-CdA, and there is no evidence in the literature to date proving the superiority of either drug.

Second-line treatment: chemoimmunotherapy should be considered

Patients who relapse after previous treatment with PNA are more difficult to treat and are at high risk of having a shorter OS. Patients who relapse after PNA therapy should be retreated with the same or the alternative PNA, depending upon the duration of remission. In the event of short-term remission (less than 5 years), chemoimmunotherapy combining PNA plus rituximab could be effective in high-risk HCL patients. In a phase 2 study, patients with previously untreated HCL (59 patients), relapsed/refractory HCL (14 patients), or HCL-V (7 patients) received 2-CdA for 5 consecutive days and rituximab (375 mg/m^2 weekly) for 8 weeks [48•] 1 month later. The complete response (CR) rates were 100%, 100%, and 87%, respectively. After a median follow-up of 60 months, the 5-year failure-free survival (FFS) was 95%, 100%, and 64%, and the 5-year OS was 97%, 100%, and 61%, respectively. MRD was undetectable in 94% of patients. Patients with HCL and unmutated *IGHV* did not experience relapse. The sequential schedule was well tolerated. Chihara et al compared the CDAR regimen combining 2-CdA (0.15 mg/kg/day) for 5 days with rituximab (375 mg/m^2 intravenous (iv) weekly) for 8 weeks (rituximab started at day 1 of 2-CdA) in 34 patients with 2-CdA plus delayed rituximab 6 months after 2-CdA if the blood MRD was positive (34 patients). The CR rate was 100% for CDAR and 88% for 2-CdA. A durable and undetectable MRD was achieved by 33/34 patients (97%) after CDAR versus in 11/34 patients (32%) after 2-CdA

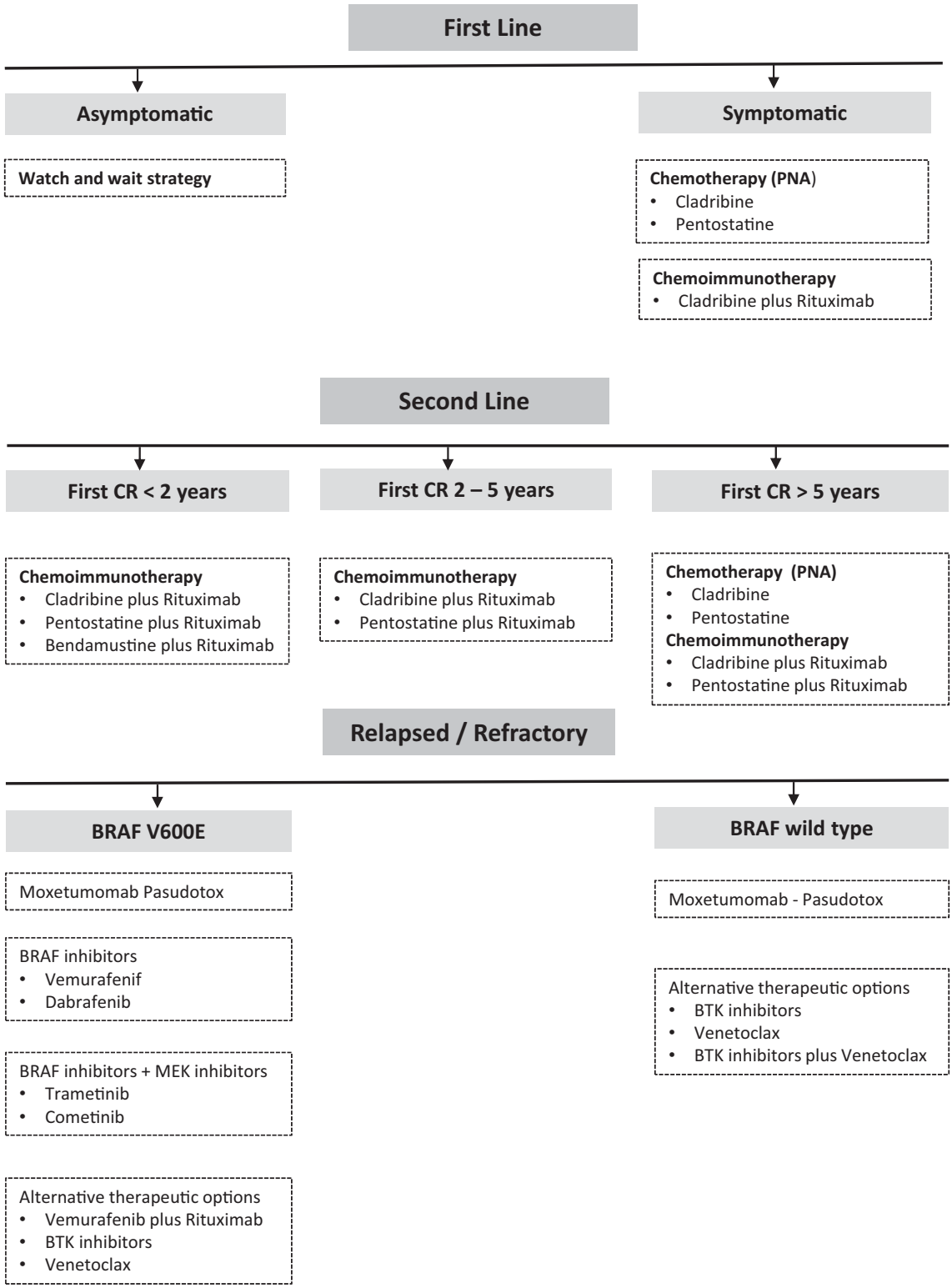

Fig. 3. Therapeutic algorithm for treatment of patients with hairy cell leukemia (HCL).

and in 14/21 patients (67%) after delayed rituximab [49]. The combination of bendamustine with rituximab could also be considered [50, 51].

Relapsed/refractory HCL patients

The three options for relapsed/refractory HCL patients include recombinant immunoconjugates targeting CD22, BRAF inhibitors, and BCR inhibitors (Fig. 3).

Immunotoxins

Immunotoxins, which are fusions of a bacterial toxin to the variable region of a monoclonal antibody that is directed against a specific cell surface target such as CD22 in HCL, represent a new therapeutic option that is now available for HCL patients (with or without *BRAF* V600E) and patients with HCL-V. The preliminary results obtained with moxetumomab pasudotox (HA22, CAT-8015) were promising in a phase 1 clinical trial in relapsed HCL patients, with an overall response rate (ORR) of 86%, including a 46% CR and no dose-limiting toxicities [52]. The results were confirmed in a phase 3 study that included 80 relapsed/refractory HCL patients (median age 60) using the drug at 40 μg/kg/j iv on day 1, day 3, and day 5, with a maximum of 6 28-day cycles. The ORR was 75%, and the CR rate was 41%, with a durable CR rate of 30%. Among patients with a CR, 85% were MRD-negative (IHC and BM). The 3 HCL-V patients did not achieve a CR. The treatment was safe, with 7.5% of patients experiencing hemolytic uremic syndrome (HUS) and 5% experiencing capillary leak syndrome [53••]. Moxetumomab pasudotox is FDA- and EMA-approved for relapsed or refractory HCL after >2 previous lines of treatment (including 1 PNA).

BRAF inhibitors

Vemurafenib as a monotherapy

There are a few reports of patients treated with vemurafenib as a monotherapy at a dose of 960 mg b.i.d. [54••, 55]. The treatment was effective, with a high rate of objective response, even if a molecular CR was not actually obtained. The treatment was well tolerated but required careful monitoring. The progression of RAS-mutant chronic myelomonocytic leukemia was reported after the initiation of vemurafenib in a patient treated for metastatic BRAF-mutant melanoma [56]. BRAF inhibition, which leads to ERK activation, can also promote chronic lymphocytic leukemia (CLL) in the absence of RAS mutations [57]. In a publication describing two studies, vemurafenib (960 mg twice daily) was administered to 54 relapsed/refractory HCL patients for 16 or 18 weeks. The ORR of the studies was 96% and 100%, with a CR in 35% and 42% of patients, respectively. With a median follow-up of 23 months, the median relapse-free survival (RFS) was 19 months for patients with a CR versus 6 months for patients with a partial response (PR). In a US study, the 1-year RFS and OS were 73% and 91%, respectively. The most frequent adverse events were cutaneous and articular. Seven patients had a second malignancy, including a patient with melanoma [54••]. Dietrich et al. [58••] evaluated the efficacy of low-dose vemurafenib. Twenty-one heavily pretreated HCL patients were treated with vemurafenib (from 240 to 1920 mg/day). The median duration of treatment was 90 days. Efficacy, toxicity, and the complete inhibition of ERK phosphorylation were observed with 240 mg given twice daily. The response rates and

kinetics were independent of the dose and duration of treatment. When progression occurred after discontinuing vemurafenib, retreatment induced a new response. Therefore, this study demonstrated the effectiveness of lower doses of vemurafenib in HCL [58••]. In 6 HCL patients with a BRAF V600E mutation, HCL patients with an active infection (both in the front-line and relapse settings) received low-dose vemurafenib (480–960 mg/day); all of these patients achieved a rapid response and recovered from their infections [59]. In a real life experience in France, 10 HCL patients with a BRAF V600E mutation were treated with vemurafenib (1920 mg/day) for 16 weeks (median age 68, median number of previous lines 3). The ORR was 100%, and the CR rate was 60%. One patient died because of HCL progression. The median duration of response (DOR) and the median progression-free survival (PFS) were 9.2 and 14.1 months, respectively [55].

BRAF inhibitors combined with MEK inhibitors

The combination of dabrafenib (150 mg b.i.d.) and trametinib (2 mg once daily), a MEK inhibitor, was evaluated in relapsed/refractory HCL. In a phase 2 trial, 43 pretreated HCL patients received continuous trametinib (2 mg once daily) and dabrafenib (150 mg twice daily) until disease progression, death, or unacceptable toxicity. Nearly half of these patients had received > 4 previous lines of treatment. The ORR was 78% (32/41 evaluable patients), with a CR rate of 49% (20/41, 6 of whom achieved a CR with MRD negativity). The 12-month PFS and OS were 98%. Forty-nine percent of patients had a grade 3/4 adverse event, and hyperglycemia, anemia, and neutropenia were especially common. Adverse events led to dose reductions in 42% of patients and were the leading cause of treatment discontinuation (5/8 patients) [60].

Vemurafenib plus rituximab

In the phase 2 HCL-PG03 trial, 31 patients (median age 59 years) with relapsed/refractory HCL received vemurafenib (960 mg twice daily) for 8 weeks with concomitant rituximab (375 mg/m^2) every 2 weeks, followed by rituximab 4 times (every 2 weeks) after finishing vemurafenib. The ORR was 100%, with a CR in 26/27 patients (96%). MRD was negative in 17/27 patients (63%). After a median follow-up of 26 months, only 4 patients relapsed, all of whom had a positive MRD and 3 of whom had previously received BRAF inhibitors as a monotherapy. Only 1/17 MRD-negative patients had a positive MRD at the end of follow-up and were still experiencing a CR at 40 months. The chemotherapy-free regimen was well tolerated and not myelosuppressive [61]. Currently, a clinical trial is recruiting previously untreated HCL patients and combines vemurafenib with obinutuzumab.

BCR pathway inhibitors

In an interim analysis of a single arm of a phase 2 study that included 28 patients (1 treatment-naïve HCL-V, 10 relapsed HCL-V, and 17 relapsed HCL patients; median age, 65; median prior therapies, 4), ibrutinib was administered at 420 mg/day (n=15) or 840 mg/day (n=13). Four patients achieved a CR, 9 patients achieved a PR and 8 patients achieved stable disease (SD). There was no difference in the response according to the dose

of ibrutinib, but the ORR was better in the patients with HCL than HCL-V, and no HCL-V patients achieved a CR. The estimated 24-month PFS was 79%. Notably, there was a durable clinical benefit even for patients with SD. In responders, there was a normalization of soluble CD25, which could be used as a biomarker for treatment efficacy. Ibrutinib was well tolerated [62].

Evaluation of the risk of secondary cancers

Patients with HCL are at risk for secondary malignancies. The long-term OS of patients with HCL must be considered, and the drugs we use must be safe and nontoxic. The occurrence of secondary cancers in HCL patients is a subject of debate [63–69]. In a retrospective survey of 487 patients with HCL, we reported a high frequency of cancers in HCL patients and their family members. Ten percent of HCL patients developed secondary malignancies after an HCL diagnosis, and 18% had a familial history of cancer. The high incidence of cancer occurring after HCL diagnosis was observed with a standardized incidence ratio (SIR) of 1.86 (95% confidence interval (CI), 1.34–2.51), and for hematological malignancies, the SIR was 5.32 (95% CI 2.90–8.92). This increased risk could be related to the disease and/or the treatment, and the respective role of DCF or 2-CdA in the development of secondary malignancies remains a topic of debate [70•].

Compliance with Ethical Standards

Conflict of Interest

Jerôme Paillassa declares that he has no conflict of interest. Xavier Troussard is a consultant for Innate Pharma.

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Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

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After analyzing the immunological profile of abnormal lymphoid cells from 194 patients with a B cell chronic lymphoproliferative disorder associated with circulating hairy or villous lymphocytes, a scoring system was proposed considering the reactivity with each of the four markers (CD11c, CD103, CD123 and CD25) and gives 1 point if positive and 0 points if negative. Scores range from 4 (typical of HCL) to 0 (atypical of HCL). 98% of HCL had high scores (3 or 4) whereas 88% of HCL-V and 77% of SLVL scored 1 or 2 and no single case scored 3 or 4.

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Hairy Cell Leukemia variant and IGHV 4-34 Hairy Cell Leukemia cases can be characterized by a high prevalence of MAP2K1 mutations and the absence of BRAF V600E mutaions.

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When selecting a panel of 21 relevant genes, BRAFV600E was found in 90% of Hairy Cell Leukemia patients and was associated with other mutations in 33% of cases. All patients with hairy Cell Leukemia Variant had mutations in epigenetic regulatory genes: KDM6A, CREBBP or ARID1A. The analysis of sequential samples (at diagnosis and relapse) showed the presence of new subclonal mutations and variations of the mutated allele frequency. The analysis opens new perspectives for personalized medicine for patients with Hairy Cell Leukemia and Hairy Cell Leukemia variant.

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In 58 patients with Hairy Cell Leukemia, the IGHV profile was unmutated in 6 patients and mutated in 52 cases. Beneficial responses were obtained with purine nucleoside analogs in 91%, whereas treatment failures were observed in 9% of cases. Failures were associated significantly with an unmutated IGHV profile, leukocytosis, and bulky spleen. The unmutated HCL not benefiting from cladribine characteristically had bulky spleen, leukocytosis and *TP53* defects, and progressed rapidly after first treatment. The data suggest that unmuttaed HC identify a minor subgroup failing cladribine treatment and with more aggressive disease.
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The study shows the presence of BRAFV600E mutation in hairy cells but also in hematopoietic stem cells of patients with Hairy Cell Leukemia. In addition, expression of BRAFV600E in murine hematopoietic/progenitor cells can cause HCL-like disease. The data demonstrate that the mature B cell malignancies can initiate in the hematopoietic stem cell compartment.
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Moxetumomab pasudotox, a recombinant CD22-targeting immunotoxin was evaluated in 80 patients with relapsed/refractory Hairy Cell Leukemia. The durable complete response (CR) rate was 30%, CR rate was 41%, and objective response rate was 75%; 80% of patients achieved hematologic remission. Among complete responders, 85% achieved MRD negativity. The treatment is likely to induce a high rate of durable response and MRD eradication in heavily pretreated patients with HCL, with acceptable tolerability.

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Of the 487 HCL patients, 18% had a familial history of cancers, 8% presented with malignancies before HCL diagnosis and 10% developed second malignancies after HCL was diagnosed. An excess incidence of second malignancies was observed, with a standardized incidence ratio (SIR) of 1·86 (95% confidence interval (CI): 1·34–2·51), with no significant difference between PNAs. For second hematological malignancies alone, the SIR was markedly increased at 5·32 (95% CI: 2·90–8·92).

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