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Diagnosis and treatment of hairy cell leukemia as the COVID-19 pandemic continues

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ABSTRACT

Hairy cell leukemia (HCL) is an indolent B-cell malignancy, usually driven by the BRAF V600E mutation. For 30 years, untreated and relapsed HCL was successfully treated with purine analogs, but minimal residual disease (MRD) remained in most patients, eventually causing relapse. Repeated purine analogs achieve decreasing efficacy and increasing toxicity, particularly to normal T-cells. MRD-free complete remissions (CRs) are more common using rituximab with purine analogs in both 1 st-line and relapsed settings. BRAF inhibitors andibrutinib can achieve remission, but due to persistence of MRD, must be used chronically to prevent relapse. BRAF inhibition combined with rituximab can achieve high MRD-free CR rates. Anti-CD22 recombinant immunotoxin moxetumomab pasudotox is FDA-approved in the relapsed setting and is unique in achieving high MRD-free CR rates as a single-agent. Avoiding chemotherapy and rituximab may be important in ensuring both recovery from COVID-19 and successful COVID-19 vaccination, an area of continued investigation.

1. Introduction to hairy cell leukemia

Hairy cell leukemia (HCL) was described in 1958 as a B-cell malignancy comprising 2% of all leukemias [1,2], which today would amount to about 1200 new cases per year in the United States [3]. In over 90% of patients with classic HCL, the disease appears to be caused by the BRAF V600E mutation, leading to constitutive phosphorylation of ERK, which in turn leads to increased proliferation [4–7]. Although difficult to prove causation, patients commonly report residential or occupational exposure to chemicals [8–11], but an association with tobacco smoking was not found [12]. While considered an indolent leukemia, in 1978 before effective systemic therapies, median survival after diagnosis was only 4 years [13]. Successful systemic treatment with interferon was a significant advance, first reported in 1984 [14]. However, the first-line treatment of HCL underwent a long-lasting major advance by the end of that decade with the discovery that purine analog pentostatin or cladribine could each achieve CR in 76–91% of patients [15–21]. Long-term follow-up documented high 5- and 10-year disease free survivals. However, a plateau on disease-free survival curves was missing, suggesting lack of cure [22,23]. According to consensus guidelines, first-line treatment of HCL includes purine analog monotherapy [24,25]. However, repeat courses of purine analog are associated not only with declining CR rates and shorter disease-free intervals, but also with increasing risk of toxicity, particularly from chronic CD4 T-lymphopenia and neuropathy [26–29]. Repeated treatments are also associated with an increased risk of secondary malignancies in some although not in all studies [30–32].

2. How to diagnose HCL

HCL patients present most commonly with fatigue (80%) and splenomegaly (80–90%), and 15–40% of patients present with fever, infections, night sweats, weight loss, left upper abdominal pain from splenomegaly, hepatomegaly, and bleeding and bruising from thrombocytopenia [33]. In 15–30% of cases, patients present with autoimmune disorders [33], including vasculitis and psoriasis [34–37]. The skin is involved in 10–12% of patients with HCL, usually due to autoimmune or infectious etiologies, and direct leukemic involvement of the skin (leukemia cutis) is much rarer [38]. Bone lesions have also been recognized as a feature of HCL [39–41]. Pulmonary abnormalities are usually due to infections or adenopathy, but leukemic involvement of the lungs was reported in 2 of 21 autopsied patients [42]. Monocytopenia is a classic feature, although HCL cells are often mistaken for monocytes based on their size [43]. The most sensitive and specific method for diagnosis is blood or bone marrow aspirate flow cytometry, which should show bright positivity for CD11c, CD22, and CD20. CD103

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is a T-cell antigen but is a specific marker for HCL if present on B-cells [33,44]. CD25 is positive and often bright in classic HCL, although it may be medium to dim. CD123 is characteristically positive in classic HCL. The most common markers by bone marrow biopsy immunohistochemistry (IHC) include CD20, also present on normal B-cells, and the HCL-specific antigens DBA44 (CD72), tartrate-resistant acid phosphatase (TRAP), annexin A1 (Anxa1), and the BRAF V600E mutation using VE1 Mab [4,33,45–48]. Double staining with Pax5 and either TRAP or CD103 is highly specific for HCL [49]. PCR techniques able to detect the BRAF V600E mutation include pyrosequencing [7], allele-specific quantitative PCR [5], and digital droplet PCR [50]. In newly diagnosed patients, the bone marrow is usually hypercellular, but patients can also present with hypoplasia and even aplasia [51]. The median age of presentation is about 55, but cytopenias are often observed for years prior to diagnosis. Besides BRAF V600E, mutated genes reported in classic HCL include cell-cycle inhibitor CDKN1B (p27), EZH2, ARID1A, KMT2C (ML3), and KLF2 [52–55]. Sixteen percent of HCL patients had mutations in CDKN1B or KLF2 which were believed to interact with BRAF V600E, causing leukemic transformation [6,56]. One classic HCL patient without BRAF V600E was described with a fusion of immunoglobulin heavy chain (IgH) with BRAF [57]. The male-female ratio is about 4, and HCL is more common in Caucasians compared with Asians, Africans, and Arabs [12]. We found that the class II human leukocyte antigen (HLA), DRB1*11, is more common in HCL compared to control Caucasian populations, and DRB1*11 is also more common in Caucasians than other populations [58].

3. How to tell HCL from variants

HCL variant (HCLv) was described in 1980 by Cawley et al. as leukocytosis more frequent than cytopenias including monocytopenia [59–62]. HCLv lacks CD25, Anxa1, TRAP, and BRAF V600E, and since it is more aggressive than HCL, in 2008 it was considered a distinct disorder by the World Health Organization [7,59,60]. By bone marrow morphology, HCLv differs from HCL in having an intra-sinusoidal appearance [45,60,62,63]. Since response of HCLv to purine analog monotherapy is poor and it requires combinations of purine analog and rituximab, an accurate diagnosis of HCLv vs classical HCL is critical [64,65]. By flow cytometry, CD11c is equally bright in HCLv and in classic HCL, but in HCLv CD25 and usually CD123 are negative [44,60,62]. Both HCL and HCLv are positive for CD103 and its absence should suggest splenic marginal zone lymphoma (SMZL) [44,66]. In the spleen, SMZL is mainly a white pulp disease while HCL and HCLv involve red pulp [45,60]. Both HCLv and the entity splenic diffuse red pulp lymphoma (SDRPL), are considered ‘splenic B-cell lymphoma/involvement red pulp’ [45,60]. Both HCLv and the entity splenic diffuse red pulp lymphoma (SDRPL), are considered ‘splenic B-cell lymphoma/leukemia, unclassifiable’ [63], with SDRPL having less anaemia, lymphocytosis, brighter CD123 and lower CD11c and CD103 expression and longer overall survival (OS) [63]. About one-third of HCLv patients express unmutated immunoglobulin rearrangement IGHV4-34 [67]. This rearrangement can also be present on HCL cells which look otherwise classic in that they are positive for TRAP and bright positive for CD25, but they lack BRAF V600E. The disease in these patients is aggressive, is associated with nodal disease and responds poorly to first line purine analog. Thus IGHV4-34+ HCL/HCLv should be considered a molecularly defined variant which overlaps with HCL [7,67–69]. A case of CLL transforming to HCLv was described in a patient with IGHV4-34+ leukemic cells [70]. While HCLv and IGHV4-34+ HCL/HCLv lacks BRAF V600E, we reported MAP2K1 mutations in about half of these patients, in addition to mutations in U2AF1, ARID1A, TP53 and TTN [71]. In several HCLv cases, Maître et al. reported mutations in KDM6A and CREBBP, which like ARID1A are epigenetic regulatory genes [53]. Other mutated or deleted genes or regions in HCLv include CCND3, CCND1 (mutation and fusion with IgH), ATM, and 7p [6,52,72,73]. Myf6 is a muscle-associated gene found to be expressed in essentially 100% of HCL classic cases and much less frequently in HCLv or IGHV4-34+ HCL [74]. Unusual markers in classic HCL like CD38 have been associated with shorter time to progression and next treatment [75]. Other aberrant markers like CD5 or CD10 have not shown prognostic implications [76–79].

4. When is treatment indicated in HCL?

A small minority of HCL patients can wait on treatment for years or decades. On the other hand, it is rare that treatment is so urgent that patients can’t wait for several weeks or months. The most commonly accepted indications for treatment are neutrophils <1–1.5 x 10⁹ cells/L, hemoglobin <10–12 g/dL, and platelets <100 x 10⁹ cells/L [17,19,25,80]. We and other groups favor the 1, 10, and 100 limits, respectively, in view of the toxicity of chemotherapy for HCL and wanting to avoid excessive retreatment [19,81–86]. Additional criteria for treatment include malignant lymphocytosis >5 or >20 x 10⁹/L or progressing lymphocytosis, symptomatic splenomegaly or hepatomegaly, enlarging lymph nodes >2cm in short axis, frequent infections, and unexplained weight loss >10% in the prior 6 months [25,87]. The additional criteria are particularly relevant for patients without cytopenias, which frequently occurs in HCL after splenectomy or in HCLv; these patients often do not qualify for clinical trials until their disease is much more advanced. In relapsed patients, it is critical to determine if cytopenias are due to progressive disease or due to toxicity from prior treatment. A common mistake is to do a bone marrow biopsy too early after treatment and treat the patient with chemotherapy if disease is detected. A patient in this situation will likely achieve CR without more treatment. This is the rationale for delaying post-treatment bone marrow biopsies until at least 4–6 months after the last treatment [25]. European Society for Medical Oncology (ESMO) guidelines recommend delaying bone marrow until after 8–9 cycles of pentostatin [88]. Nevertheless, it may still be challenging to determine whether residual cytopenias and disease by bone marrow mean that treatment is required, or whether cytopenias are due to treatment toxicity and will resolve. For patients like this, flow cytometry of the blood at several time points can be useful; an increasing HCL count suggests that cytopenias are related to residual disease. On the other hand, undetectable HCL cells in blood often indicates a good response to the last treatment and suggests that cytopenias will resolve with additional time without further treatment.

5. How response to treatment is determined in HCL

5.1. Criteria for complete remission (CR)

Response criteria by the 1987 Consensus Resolution [89] were upheld 30 years later by the 2017 consensus guidelines [24,25] in requiring elimination of HCL cells by non-immunologic Wright stain and hematoxylin/eosin (H/E) stain of bone marrow and blood. Criteria for resolution of cytopenias required neutrophils, hemoglobin and platelets at least 1.5 x 10³, 11–12 g/dL, and 100 x 10⁹/L respectively [25,81–86,89,90]. When iron deficiency is present, we often overlook the hemoglobin since deficient iron stores will limit recovery of erythrocytes. While consensus guidelines allow physical exam to document resolved splenomegaly, registration (pivotal) trials leading to FDA approval require objective documentation, and ultrasound, which is an operator dependent, is insufficient. Rather, CT or MRI have to show resolution of cytopenias required neutrophils, hemoglobin and platelets at least 1.5 x 10³, 11–12 g/dL, and 100 x 10⁹/L respectively [25,81–86,89,90]. When iron deficiency is present, we often overlook the hemoglobin since deficient iron stores will limit recovery of erythrocytes. While consensus guidelines allow physical exam to document resolved splenomegaly, registration (pivotal) trials leading to FDA approval require objective documentation, and ultrasound, which is an operator dependent, is insufficient. Rather, CT or MRI have to show resolution of cytopenias required neutrophils, hemoglobin and platelets at least 1.5 x 10³, 11–12 g/dL, and 100 x 10⁹/L respectively [25,81–86,89,90]. When iron deficiency is present, we often overlook the hemoglobin since deficient iron stores will limit recovery of erythrocytes. While consensus guidelines allow physical exam to document resolved splenomegaly, registration (pivotal) trials leading to FDA approval require objective documentation, and ultrasound, which is an operator dependent, is insufficient. Rather, CT or MRI have to show resolution of cytopenias required neutrophils, hemoglobin and platelets at least 1.5 x 10³, 11–12 g/dL, and 100 x 10⁹/L respectively [25,81–86,89,90].
as 0.002% HCL cells [92]. Bone marrow aspirate flow cytometry is most sensitive by far for MRD [65,87,93,94]. Allele-specific PCR for the BRAF mutation is sensitive to 0.1–0.05% [5,95], about 25-fold less sensitive than flow cytometry, but BRAF PCR is still more sensitive than PCR to detect monoclonality of the immunoglobulin rearrangements [64,87]. IHC-MRD criteria since 1999 require at least as many B-cells as T-cells, and require most of the B-cells (CD20+ or DBA44+) to be consistent with HCL [96].

5.3. Criteria for partial response (PR)

By consensus guidelines [25,88], PR requires ≥50% reduction in palpable spleen, liver and lymph node size, and resolution of cytopenias to levels required by CR. Others as well as our group at NIH consider PR if there is ≥50% improvement in neutrophils, hemoglobin and platelets [19,81–86,90,97]. Among partial responders in these protocols, those who do meet the CR criteria with respect to resolution of cytopenias are considered a ‘good PR’ (GPR) [90] or hematologic remission (HR) [85,86,91,94]. Those who are red cell transfusion dependent at baseline require a hemoglobin improving to 9.0 g/dL, which would be a 50% improvement over a baseline hemoglobin of 6.0 g/dL. However, a PR cannot start until ≥4 weeks after last blood transfusion or growth factor [86]. For both PR and CR, the minimum duration of resolved cytopenias, not specified in the guidelines [25], is 4 weeks in several protocols [17,85,86,90,91,93,94]. We do not require a 4-week duration of resolved cytopenias for 1st or 2nd-line HCL treatment, since improvements are more durable after therapy of early HCL [87,93]. The older criteria for PR which required ≤5% circulating HCL [89] is no longer required for PR [25]. However, many protocols including ours require ≥50% reduction in circulating HCL cells [19,25,98]. Consensus guidelines for PR have required ≥50% reduction in HCL infiltration in bone marrow biopsy [25,89]. We have avoided doing bone marrows just to document PR in patients without blood counts consistent with CR. Percent marrow infiltration can be an inaccurate measure of response due to marrow heterogeneity, which is particularly common in multiply relapsed HCL. Consensus guidelines for PR require ≥50% reduction in abnormal hepatosplenomegaly at least by exam, while other protocols including ours require CT/MRI of spleen and lymph nodes to document ≥50% improvement or resolution to CR levels [91,94,99]. Even with no residual HCL, splenomegaly can persist due to benign elements and the associated hypersplenism can cause thrombocytopenia. Splenectomy should be avoided for these patients [100]. Flow cytometry of the blood and assays of soluble forms of CD25 or CD22 [101] may be useful assessing residual HCL burden with splenomegaly. On the other hand, Residual lymph nodes >2 cm in short axis can often occur due to persistent disease, and a PET scan or nodal biopsy can be helpful in these cases.

5.4. Criteria for progressive and stable disease

By consensus guidelines, progressive disease (PD) requires ≥25% worsening of cytopenias not due to toxicity, or ≥25% worsening of hepatosplenomegaly [25]. Other protocols including ours have included ≥50% increase in hepatosplenomegaly, ≥50% increase in circulating HCL cells, or ≥25% increase in adenopathy [91,93,102]. Patients not meeting criteria for CR, PR or PD are considered to have stable disease (SD).

6. First-line treatment of HCL

6.1. First-line purine analog monotherapy

For the past >30 years, purine analog monotherapy has been considered the treatment of choice for HCL in 1st line. While never compared head-to-head, pentostatin and cladribine are considered similar with respect to efficacy and toxicity, both achieving CR rates of 70–90% and treatment-free durations >10 years [17,19,21,103–105]. Either can cause neutropenia and fever, mostly during the first month, particularly in patients with severe baseline neutropenia, but even in patients with neutrophil counts >1 × 10⁹/L at baseline. Cladribine rashes are common [106,107]. Reductions in CD4+ T-cells have been described with both agents lasting a median of 40–52 months [26,27]. Neutropenia is seen in ~15% of patients after one course and can be both cumulative and delayed [19,29,90]. Cladribine became much more commonly used than pentostatin since it is administered over 5–7 days, while pentostatin is given every 2 weeks for 3–6 months. However, pentostatin may be preferable in high-risk patients who could use reduced doses initially and then return to full doses for later cycles [25]. On the other hand, cladribine can be given weekly, usually for 6 doses, to allow more gradual administration [108–110]. Two clinical trials randomized 100 and 132 cladribine-naive HCL patients: 1:1 to 5 daily vs 6 weekly doses of cladribine and showed similar efficacy without safety advantages to the weekly schedule [111,112]. Cladribine is also effective subcutaneously and pharmacokinetic studies have documented adequate delivery [113–117]. When followed primarily by blood counts, patients relapse from CR at a median of ~15 years after 1st-line purine analog [21]. Of 208 patients treated with cladribine (n = 159) or pentostatin (n = 49), the median relapse-free survival was 11 years [32]. However, patients up to 40 years of age, followed by bone marrow, were reported to relapse in <5 years [118]. There is no evidence showing plateau long term in failure-free survival to suggest cure for HCL patients [19,21,104,105]. Of the original 358 patients treated with cladribine at Scripps [19], 19 who were in continuous remission for a median of 16 years were studied, and bone marrow studies showed relapse in 3, MRD in 7, and MRD-free CR in 9 [119]. In 34 patients randomized to cladribine alone, only 4 (12%) of the patients were in MRD-free CR at a median follow-up of 6.5 years [87]. Thus, cure after purine analog monotherapy, if possible, is unlikely at best.

6.2. Cladribine with rituximab in first line therapy of HCL

Ravandi et al. reported rituximab in 8 weekly doses beginning 1 month after cladribine for 1st line HCL treatment. The CR rate was 100% of 36 patients [64]. At the 3-month time point after cladribine, 22 (79%) of 28 evaluable patients were MRD-free by bone marrow aspirate flow cytometry. An update of this data reported that the CR rate was still 100% in 59 1st-line patients, 76% achieved MRD-free CR, 4 (7%) of the patients became MRD+ in follow-up, and 2 patients required next treatment [120]. In this trial, persistent disease in the bone marrow was observed in 44% of 36 patients prior to rituximab, compared to 0% at 6 months after cladribine or 3 months after rituximab [64]. However, since many patients achieve CR 4–6 months after cladribine, the effect of rituximab could not be determined without a control group. We therefore performed a randomized trial of cladribine with rituximab begun on day 1 of cladribine or delayed at least 6 months later until MRD was detected in blood. Because rituximab was begun immediately rather than at 1 month, cladribine-rituximab (CDAR) synergy was possible based on the hypothesis that rituximab increases cell sensitivity to purine analog [121,122]. Since purine analogs are cleared within 1–2 days, this type of synergy is not possible with delayed rituximab. At the 6-month time point, which was before delayed rituximab could be given, the CR rate was similar for CDAR, 34 (100%) of 34, and for cladribine monotherapy, 31 (91%) of 34, (p = 0.24), but MRD-free CR was greatly improved, 33 (97%) vs 8 (24%) (p < 0.001). Moreover, as shown in Fig. 1A, of 11 patients achieving MRD-free CR with cladribine alone, including 3 who became MRD-free after the 6-month time point, only 4 (12%) remained MRD-free at 6.5 years of median follow-up, compared to 32 (94%) of patients with CDAR (p < 0.0001). Thus, immediate rituximab greatly improved the MRD-free CR rate and duration after cladribine. However, we found that delayed rituximab after cladribine alone achieved MRD-free CR in 14 (67%) of 21 patients. Fig. 1B
6.3. First-line therapy of HCLv

HCLv responds poorly to purine analog monotherapy and OS is 6–9 years compared to >25 years for classic HCL [61–63,65,67,125,126]. Of 42 historical HCLv patients who received cladribine monotherapy, the CR rate was only 7% [20,126–130]. At MD Anderson Cancer Center, 7 HCLv patients received 1st-line treatment with cladribine followed 4 weeks later by 8 weekly doses of rituximab with a CR rate of 86% and MRD-free CR rate of 71% [54,120]. Two MRD-negative HCLv patients became MRD+ and the 5-year FFS and OS were 64.3% and 51.4%, respectively, at a median follow up of 60.2 months. OS was adversely affected by secondary malignancies [64,120]. We reported phase 2 data for CDAR in patients with HCLv [65] [131], with a CR rate of 95% and MRD-free CR rate of 80% by bone marrow aspirate flow cytometry. Failure of CDAR to achieve MRD-free CR by 1 or 6 months, or the presence of TP53 mutations, was associated with decreased progression-free survival (PFS) and OS [131]. Based on these data, patients with HCLv should not be treated with purine analog monotherapy. We have also found that patients with unmutated IGHV4-34+ HCL respond poorly to purine analog monotherapy but, like HCLv, respond well to combinations of purine analog and rituximab [67,132]. These cases are generally BRAF wild type [7], and it has been reported that BRAF wild type HCL not responding to purine analog monotherapy can respond well to purine analog-rituximab combinations [133]. Compared to CDAR, combinations of bendamustine-rituximab [93] and pentostatin-rituximab are potentially more synergistic because more doses of rituximab are combined with purine analog. Therefore, we are testing bendamustine-rituximab vs pentostatin-rituximab in HCLv in both 1st-line and later-line settings at NIH. This trial, along with other HCL/HCLv clinical trials listed on the HCL Foundation website, is shown in Table 1 and Fig. 3.

7. Second-line treatment of HCL.

According to 2017 consensus guidelines, patients who relapse ≥2 years after first line purine analog monotherapy can be retreated with the same or alternate purine analog. Second-line purine analog therapy achieves CR rates of 62–69% [19,21]. By bone marrow, 2nd response duration is as short as 23 months [19]. In patients followed primarily by blood counts, median PFS of 9 years has been reported for 2nd-line purine analog [21]. In general, repeated courses of purine analogs lead to decreasing efficacy and increasing cumulative toxicities, and the 2021 NCCN guidelines now list purine analog + rituximab rather than purine analog monotherapy for 2nd line treatment [124]. Several studies have shown superior CR rates and durability after purine analog-rituximab in 2nd line compared to the same purine analog given as monotherapy during 1st-line [105,120,134]. Of 14 patients receiving cladribine followed 4 weeks later by rituximab in 2nd-line, the CR rate was 100%, and MRD-free CR rate was 64% [120]. Cladribine with immediate vs delayed rituximab is currently being evaluated at NIH for patients with one prior course of purine analog (Table 1, Fig. 3). In the 2021 NCCN guidelines, rituximab alone is listed as an option for patients who are intolerant of purine analog [124]. As a single-agent, rituximab achieved CR rates of 10–55% in 6 studies with a total of 97 patients [135–140], but of 51 patients with at least one prior purine analog and cytopenias requiring treatment, the CR rate was 20%. In the largest study with such patients, the CR rate was only 13% [137], indicating that the activity of rituximab monotherapy in relapsed HCL is limited. Interferon can achieve responses in HCL [141], but in the randomized trial with pentostatin, no patients crossing over from pentostatin to interferon achieved CR [17]. For patients with <2-year response to 1st course of purine analog, NCCN guidelines now list vemurafenib [124]. The remainder of this review will focus on targeted therapies for multiply relapsed HCL, including recombinant immunotoxins targeted to the cell surface, one of which is FDA-approved for HCL.
8. Recombinant immunotoxins

8.1. Definition of recombinant immunotoxin

Recombinant immunotoxins are chimeric proteins engineered to contain a protein toxin fused to a monoclonal antibody fragment [142]. Like antibody drug conjugates (ADC), they bind to cell surface receptors or antigens which internalize them, and the internalized molecule induces cell death. Unlike ADCs, where the toxic payload is a chemotherapeutic agent that the tumor may be resistant to, in recombinant immunotoxins the killing agent is a protein toxin that kills by arrest of protein synthesis due to ADP-ribosylation and inactivation of elongation factor-2 (EF2) [143,144]. Protein synthesis arrest is associated with a decrease in MCL-1, and activation of BAK and the apoptotic cascade [145]. Plant toxins like ricin, which arrest protein synthesis by ribosomal inactivation, have been used to make chemical immunon conjugates, including one targeting CD22 [146,147]. Bacterial toxins like Pseudomonas exotoxin A (PE) and diphtheria toxin (DT) are advantageous for making recombinant toxins, since they are naturally made as single chains [148]. Humans are vaccinated against DT but not PE, which comes from Pseudomonas aeruginosa, so few patients have pre-existing antibodies able to neutralize PE-containing recombinant immunotoxins. These molecules are believed to kill by binding to host cells, unfolding in acidic endocytic vesicles [149], undergoing furin proteolytic cleavage in the translocating domain between the ligand and toxin [150–152], undergoing disulfide bond reduction liberating the toxin from the antibody fragment [153], trafficking the ADP-ribosylating toxin fragment to the endoplasmic reticulum [154], translocating to the cytosol [155,156], ADP-ribosylating EF2 [157,158] and inhibiting protein synthesis, leading to apoptotic cell death [145,159–161].

8.2. LMB-2 targeting CD25

Originally called anti-Tac(Fv)-PE38, LMB-2 contained a single-chain Fv form of anti-Tac targeting CD25, connected to a 38 kDa truncated form of PE called PE38 [162–167]. In phase 1 trials of B- and T-cell tumors, HCL responded best with 3 PRs and 1 CR out of 4 patients [81,82].

8.3. Anti-CD22 recombinant immunotoxins for HCL

Since CD22 greatly outnumbers CD25 in classic HCL, and HCLv as well as other B-cell malignancies express CD22 without CD25 [168], anti-CD22 immunotoxins containing truncated PE were engineered, starting with chemical conjugates [169,170]. The recombinant immunotoxin BL22 was engineered containing a disulfide stabilized Fv fragment binding to CD22 and connected to PE38 [168,171–173]. BL22 achieved CR rates of 47–61% in phase 1 and 2 HCL clinical trials [83–85]. An important toxicity observed was hemolytic uremic syndrome (HUS), not observed with LMB-2 or other recombinant immunotoxins containing PE38. It was completely reversible, occurred in 5–12% of HCL patients, and did not require plasmapheresis for complete resolution, suggesting a mechanism different from Shiga-like toxin-induced HUS [174].

8.4. Engineering of moxetumomab pasudotox

To increase the activity and specificity of BL22, hot-spot mutagenesis created mutations in the complementarity determining region 3 (CDR3) domain of BL22 which were screened by phage display selection. A mutant containing THW instead of SSY at positions 100, 100a and 100b of the VH had 14-fold improved CD22 binding affinity, mainly due to slower off-rate [175]. The higher binding affinity led to a several-fold improvement in cytotoxicity toward both CLL and HCL. The mutant recombinant immunotoxin was first called HA22, then CAT-8015, and finally moxetumomab pasudotox (Moxe). Preclinical antitumor experiments showed improved activity compared to BL22 [176], and phase 1 clinical testing began May, 2007.

8.5. Moxe phase 1 testing, dose escalation

In the initial phase I report, 16 patients received 5–40 μg/kg every other day for 3 doses (QOD x3) per 28-day cycle, and 12 received 50 μg/kg [86]. There were 13 (46%) CRs, 10 (77%) of which remained in CR at a median of 29 months. The CR rate was not related to the number of prior courses of purine analog, but those with prior splenectomy (N = 7) had no CRs, while 13 (62%) with spleens up to 325 mm had CR (p = 0.007). This suggested that patients who are post-splenectomy have more advanced disease because they require more tumor infiltration into bone marrow to cause cytopenias severe enough for eligibility. For this reason, patients should receive Moxe prior to splenectomy. Grade 2 (moderate) HUS was documented in 2 patients (one at 30 and one at 50 μg/kg), limited to grade 1 thrombocytopenia and grade 1 creatinine elevations. These mild reversible laboratory abnormalities might not have been detected had patients not been watched for HUS. Grade 3–4 toxicities included grade 3–4 lymphopenia and leukopenia, probably representing treatment effect due to targeting leukemic and normal CD22+ B-cells. Common grade 1–2 toxicities included hypalbuminemia, weight gain, edema, and proteinuria due to capillary leak syndrome (CLS). Transaminase elevations not impairing hepatic function were observed and were not considered dose-limiting per protocol.

See Fig. 3 for abbreviations. For more information on trials, see the Hairy Cell Leukemia Foundation Website www.hairycellleukemia.org/clinical-trials-patients

Table 1

Clinical trials enrolling for HCL/HCLv from Hairy Cell Leukemia Foundation website.

| Agent(s) Route | Disease | treatment line | Target or antigen | Chemo therapy | Location |
|---------------|---------|----------------|-------------------|---------------|----------|
| Cladribine-Rituximab iv | HCL | 2nd line | CD20 | Yes | NIH |
| Pentostatin-Rituximab vs iv | HCL/HCLv | ≥2–3rd line | CD20 | Yes | NIH |
| Bendamustine-Rituximab iv | HCLv | ≥2nd line | CD22 | No | NIH |
| Moxetumomab pasudotox IV | HCLv | ≥3rd line | CD22 | Yes | NIH |
| Blinatumomab Oral T-cells (CAR-T) IV | HCLv | ≥3rd line | CD22 | Yes | NIH |
| Encafonenib-Binimetinib Oral | HCL | ≥2–3rd line | BRAF | No | NIH |
| Binimetinib Oral | HCL/HCLv | ≥2–3rd line | MEK | No | NIH |
| Vemurafenib Oral | HCL | 1st line | BRAF & CD20 | No | MSKCC |
| With Obinutuzumab IV |  |  |  |  |  |
| Vemurafenib-Cobimetinib Oral | HCL | ≥3rd line | BRAF-MEK & CD20 | No | Italy |
| And/or Obinutuzumab  |  |  |  |  |  |

See Fig. 3 for abbreviations. For more information on trials, see the Hairy Cell Leukemia Foundation Website www.hairycellleukemia.org/clinical-trials-patients
8.6. Phase 1 efficacy of Moxe at the expansion dose level

The phase I study enrolled 21 more patients at 50 μg/kg, making a total of 33 at 50 μg/kg and 49 patients at all doses, without DLT or additional HUS cases [94]. Once achieving MRD-free CR including by bone marrow aspirate flow cytometry, patients could receive 2 additional (consolidation) cycles. Fig. 2 shows response in the first patient at 50 μg/Kg QOD x3, who remains in MRD-free CR at the 12.5-year time point after CR. Of 33 patients at 50 μg/kg QOD x3, 21 (64%) achieved CR with ORR 88%. Of 20 CRs evaluable for MRD by all tests including bone marrow aspirate flow cytometry, 11 (55%) achieved MRD-free CR with only one relapse, and the other 10 remained in CR up to 72 months, with a median of 42 months. In contrast, of 9 patients with MRD + CR, 8 relapsed, so median CR duration was significantly longer for MRD-free vs MRD+ CR (not reached vs 13.5 months p < 0.0001). This was to our knowledge the first report that eradication of MRD in HCL is associated with longer CR duration, but there have been more reports since. The duration of MRD+ CR after ≥3rd line treatment is much shorter than after 1st-line HCL treatment with cladribine [87], suggesting it is particularly important to eliminate MRD in multiply relapsed HCL.

8.7. Pharmacokinetics and antidrug antibodies against Moxe

Despite the immunogenicity of the bacterial toxin in Moxe, only 1 of 28 evaluable patients had high levels of neutralizing antibodies after cycle 1 [86]. At 50 μg/Kg, 29 patients were evaluable for pharmacokinetic assays. Patients usually had low levels of Moxe after the 1st dose because high CD22 density on HCL cells caused a CD22-sink effect. Because Moxe rapidly reduced tumor burden between the 1st and 3rd doses of cycle 1, peak levels and areas under the curve (AUC) increased, while volumes of distribution (Vd) and clearance decreased. In assays of antidrug antibodies (ADA), 15 evaluable patients had over 50% neutralization of 200 ng/ml of Moxe before cycles 2 (n = 3), 3 (n = 5), 4 (n = 4), 5 (n = 2) and 6 (n = 1), and hence became ineligible for retreatment. However, they did receive the cycle after the ADA assay because ADA levels were not known until after the cycle was completed. We found that peak levels and AUC increased while Vd and clearances decreased between days 1 and 5 of nearly all these final cycles [94]. Thus, repeated doses of Moxe titrated out neutralizing antibodies and even the secondary immune response was inadequate in these patients to prevent therapeutic plasma levels of Moxe. In fact, 9 of these 15 patients achieved CR. Moreover, patients did not have side effects during production of ADA. These data justified retreatment with Moxe regardless of ADA and justified enrolment and retreatment during phase 3 pivotal testing without testing ADA.

8.8. Pivotal phase 3 testing of Moxe

Moxe was evaluated in an international phase 3 trial at 32 centers in 14 countries in relapsed/refractory HCL [91]. Patients required ≥2 prior treatments including ≥1 purine analog, and either rituximab, BRAF inhibitor or a 2nd course of purine analog. Need for treatment included at least one cytophenia or painful spleen. A total of 80 HCL patients received Moxe at 40 μg/kg QOD x3 with cycles 28-days apart. Patients had to stop earlier than 6 cycles in the event of MRD-free CR, progressive disease, or unacceptable toxicity. Since MRD-free CR would make the patients ineligible for retreatment, we did not restage patients until after the maximum 6 cycles. The 50 μg/Kg dose level during phase 1 was similar to 40 μg/kg during phase 3 due to improvements in purity. The primary endpoint was to achieve at least 28% of patients with ‘durable CR’, defined as CR followed by continued hematologic remission (HR) for over 180 days. Eighty patients were enrolled, ages were 34–84 (median 60) years, number of prior treatments were 2–11 (median 3), 18% had a BRAF inhibitor, 75% had rituximab, and 29% had purine analog-rituximab combinations [91]. Fifty (63%) patients completed all six cycles and 12 (15%) stopped early due to MRD-free CR, while 12 (15%) stopped early due to an adverse event which was treatment-related in 8 (10%) patients. At a median duration of follow-up of 24.6 months, the CR rate was 41% (33 of 80), ORR was 75%, and durable CR rate was 36% (29 of 80) [99]. Of the 4 patients who achieved CR but not durable CR, only 2 had recurrent cytophenias during the 180-day follow-up period, while 2 decided to travel and could not complete the monthly blood counts. Of the 33 CRs, 27 were MRD-free by IHC, and only 7 of these 27 relapsed, while 4 of 6 MRD+ CRs relapsed. Among the 6 patients with IHC-positive CR, median CR duration was 5.9 months vs not reached in the 27 who were MRD-free by IHC. Grade 3–4 toxicity occurred in 16.3% of the 80 patients, and lymphopenia (30%), HUS (5%), infection (2.5%), and CLS (2.5%) were observed. All CLS and HUS cases were reversible. There were 3 (3.8%) patients who died of infection, none Moxe-related. ADA were determined by ELISA assay [177] and ADA at baseline always involved antibodies binding to Moxe toxin domains [91]. Patients with CR had lower levels of ADA than those with PR, followed by stable disease and progressive disease [91]. ADA titers >10,240 were associated with higher clearances. Clinical benefit from Moxe was observed even in patients with low exposure or high ADA [178]. Based on the phase 1 and 3 data, Moxe was approved by the FDA in 2018, with the name Lumoxit, indicated for patients with relapsed/refractory HCL after ≥2 prior systemic therapies, at least one of them a purine analog.

8.9. Prevention of CLS and HUS from Moxe

In phase 3 testing, CLS and HUS occurred in 8.8% and 7.5% of...
patients respectively [91,99]. HUS in 7 HCL patients after BL22 and in 6 HCL patients after Moxe always presented on day 7 or 8, which is 2–3 days after the last dose of Moxe, and never presented after day 8 [83–86,91,94]. Accordingly, we check labs on day 8 looking for increased creatinine, lactate dehydrogenase (LDH), and indirect bilirubin, and decreased hemoglobin, platelets, and haptoglobin. On the phase 1 trial, abnormal creatinine and platelets were limited to grade 1 severity, and either alone would not be considered HUS. It is possible that Moxe at high concentration binds to glomerular endothelium, possibly to an antigen other than CD22, exposing ultra-large multimers of von Willebrand’s factor under the endothelial surface, which causes platelet aggregation and thrombin formation [174]. The hypothesis for Moxe binding to a glomerular antigen other than CD22 is based on lack of CD22 in that tissue and because HUS was not more common with Moxe than with BL22, despite the 14-fold improved binding affinity of Moxe vs BL22 for CD22 [175]. The movement of fluid and protein out of the blood vessels occurring in CLS can cause intravascular volume depletion and hence an increase in Moxe concentration in the glomerular capillaries, increasing the risk of HUS. To prevent HUS, we keep the concentration of Moxe in the glomerulus low using oral hydration. While too much IV fluid leads to systemic and pulmonary edema, oral hydration is more rapidly regulated and prevents hypovolemia without worsening CLS. Since the capillary leaking is constant, we encourage patients to drink an average of 1 cup (250 ml) of water per hour from day 1 to 8 of each Moxe cycle. Patients should not go more than 3 h at night without drinking. This requires patient education and monitoring [179] but it is well tolerated without even causing hypotremia since the hydration is gradual. Headache, nausea and fever which can happen 6–24 h after Moxe, can prevent patients from drinking, and these symptoms can be treated with dexamethasone 4 mg orally. Before these precautions using oral hydration and dexamethasone were instituted, 3 of 9 phase 3 patients had grade 3 HUS, vs only 1 case of grade 1 HUS in the next 17 patients (p = 0.032 for grade 3 HUS). We have adopted these precautions to prevent toxicity from Moxe and permit more cycles of treatment to safely achieve MRD-free CR.

8.10. Further development of Moxe for HCL

In a recent report, 3 patients were retreated with Moxe, two of them achieving CR and PR [180]. The responding patients had low levels of neutralizing antibodies from the phase 1 trial, were negative 12 years later prior to Moxe retreatment and became ADA-positive after retreatment. To increase the chance of MRD-free CR with Moxe, we are now testing Moxe with rituximab (Table 1, Fig. 3). The rationale is that 1) rituximab depletes circulating B-cells for about 6 months after last dose, which may prevent ADA and increase plasma Moxe levels, and because 2) rituximab can reduce HCL volume, permitting Moxe to reach more HCL cells and hence achieve CR earlier. Although rituximab failed to prevent immunogenicity of LMB-1 targeting solid tumors [181], it may prevent ADA in HCL since such patients have low humoral immunity compared to those with solid tumors. Also, LMB-1, unlike Moxe, is a chemical conjugate containing murine IgG constant domains [182]. In the phase 1 trial of Moxe-rituximab, the rituximab begins on day -2, 3 days before Moxe which is given days 1, 3 and 5. This allows rituximab time to clear normal B-cells and HCL burden prior to the 1st dose of Moxe. On retreatment cycles spaced 28 days apart, rituximab can be given just prior to Moxe on day 1. Despite the above-mentioned marginal activity of rituximab monotherapy in relapsed HCL [137], we reported that concurrent rituximab improves the MRD-free CR rate 6 months after cladribine from 24% to 97% [87]. This suggests that Moxe, which has more single-agent effectiveness than cladribine in achieving MRD-free CR, might be improved with rituximab. Rituximab was also able to dramatically improve the MRD-free rate of vemurafenib from 0% [98], to 57%, as reviewed below [95,183]. The Vemurafenib-Obinutuzumab combination is currently being tested in 1st-line (Table 1, Fig. 3) and we believe Moxe-rituximab or Moxe-Obinutuzumab should be tested in 1st line for HCL. Vemurafenib and other targeted agents with activity in HCL will now be reviewed.

9. Targeting the BRAF pathway in HCL

9.1. BRAF in HCL and single agent targeting

The RAF-MEK-ERK signaling pathway mediates proliferation, differentiation, apoptosis and development in many cell types [184]. In 2011, Tiacci et al. reported that the BRAF V600E mutation, already observed in half of malignant melanomas, was detectable in nearly all classic HCL patients [4]. In the RAF-MEK-ERK pathway, BRAF V600E results in constitutive phosphorylation of MEK1 (encoded by MAP2K1), which then activates ERK by phosphorylation, leading to malignant proliferation [4,6,98]. Besides HCL and melanoma, other hematologic disorders carrying the V600E mutation include multiple myeloma [185], Langerhans cell histiocytosis [186], and Erdheim-Chester Disease [187]. BRAF lacks V600E (wild-type, WT) in patients with HCLv, unmutated IGHV4-34+ HCL, and some other classic HCL patients [7,56,188]. Vemurafenib, which has specificity for the BRAF V600E mutation and achieves improved OS in melanoma [189], was reported to have clinical activity in HCL [190]. Clinical trials in Italy and in the US showed 35–42% CRs and ORR 96–100% [98]. Vemurafenib at 960 mg twice daily was administered for 8 weeks and was extended to 16–20 weeks if CR was not achieved by 8 weeks. Common toxicities were dermatologic and joint-related, including skin cancers. Responses occurred rapidly, particularly resolution of cytophenias [98,190]. The median relapse-free survival in the Italian trial was 9 months for responders and 19 months for CRs, which were always MRD+ by BM/Bx IHC [98]. In a retrospective trial, reduced dose vemurafenib at 240 mg/day was effective in HCL with resolved cytophenias in 21 patients [191]. At 480 mg/day, vemurafenib showed complete inhibition of signal-regulated kinase phosphorylation, and CR was achieved in 15 (40%) of 35 evaluable patients. Toxicities were still observed along with paradoxical ERK activation in BRAF WT cells [192]. Vemurafenib has been used as bridging therapy prior to definitive first-line therapy of HCL due to its rapid reversal of cytophenias, vemurafenib was useful in a Jehovah’s Witness HCL patient [192], as well as 22 patients with deep neutropenia and high risk of toxicities to cladribine [193]. This strategy has been used during the COVID-19 pandemic to delay definitive immunosuppressive therapy in patients who would be at risk of adverse outcomes if infected with COVID-19 [192–194]. The BRAF V600E inhibitor dabrafenib achieved CR a patient with both HCL and melanoma...
Dabrafenib monotherapy, in a single-center pilot phase 2 trial in 10 patients with relapsed/refractory BRAF V600E+ HCL, achieved 3 (30%) CRs and 5 (50%) PRs for an ORR of 80% [196]. All 3 CRs had MRD by IHC, and one remained progression-free at 60.5 mo while the other progressed at 15.5 and 14, requiring therapy at 31 and 21 months. Patients had received dabrafenib for 12 weeks except for 1 CR who received 8 weeks of dabrafenib. Toxicities included arthralgias, facial flushing, warts, asymptomatic QTc prolongations, and elevations of hepatic and pancreatic enzymes. Fevers were not reported during the short course of treatment.

9.2. Targeting BRAF V600E and MEK

In melanoma, the combination of dabrafenib and the MEK inhibitor trametinib achieved longer median OS as compared with vemurafenib monotherapy, without an increase in toxicity, and in fact squamous cell cancers of the skin and keratoacanthomas were less frequent with dabrafenib-trametinib (1%) compared to vemurafenib (18%) [197]. The lower incidence of skin toxicity is consistent with downregulation by trametinib of the paradoxical activation of the MAPK pathway [197]. An international multicenter trial of dabrafenib and trametinib has completed accrual in HCL and other BRAF V600E+ histologies, and efficacy in anaplastic thyroid cancer led to FDA approval of the combination in that disease [198]. In HCL, dabrafenib-trametinib had significant clinical activity as reported at the 2018 American Society of Hematology Meeting. The combination of vemurafenib and cobimetinib is being tested in Europe, and the BRAF-MEK inhibitor combination encorafenib-binimetinib, approved for melanoma [199], is being tested in HCL patients at NIH (Table 1, Fig. 3). Patients WT for BRAF are generally ineligible for BRAF inhibitors but nevertheless may respond to MEK inhibition with trametinib monotherapy [200]. MEK inhibition with cobimetinib was reported to be effective in a patient resistant to vemurafenib because of reactivation of MEK-ERK signaling [201]. The MEK inhibitor binimetinib is currently being tested in patients with BRAF WT HCL and HCLv at NIH (Table 1, Fig. 3).

9.3. Combined treatment with BRAF inhibition and CD20 Mab to eliminate MRD

Elimination of MRD with rituximab has been reported when used alone [87,202,203] or when combined with purine analogs [21,64,87,105,120,134,204]. To eliminate the MRD remaining with vemurafenib, 30 patients with relapsed/refractory HCL received vemurafenib 960 mg twice daily for 8 weeks with rituximab every other week for 16 weeks, including a 2-week break for restaging after the vemurafenib [95]. The CR rate was 87%, and the MRD-free CR rate was 57%. MRD in this study was detected by allele-specific PCR for the BRAF V600E mutation, which was sensitive to 0.05%. At a median of 34 months, of 26 patients with CR, 4 of 9 MRD−CRs relapsed vs 0 of 17 MRD-negative CRs. Observed toxicities were as expected for each agent and no new safety signals for the combination were observed. An abstract with this data was published in 2019 183 before the COVID-19 pandemic. Thus, the potential of rituximab to interfere with immune response to either COVID-19 infection or vaccine was not a factor in accrual to this trial. A phase 2 trial combines obinutuzumab and vemurafenib for first line treatment of HCL, and this trial was interrupted somewhat by the COVID-19 pandemic (Table 1, Fig. 3). Recently, 4 patients were reported who received vemurafenib and rituximab after prior Moxe [205]. One of these 4 patients achieved an MRD-free CR ongoing at 38 months, one relapsed from MRD-free CR at 13 months with massive splenomegaly and pancytopenia and could not achieve a 2nd MRD-free CR with additional vemurafenib-rituximab, one had a hematologic remission for 18 months, and one died 3 weeks after vemurafenib with pneumonia and septic shock [205].

10. Inhibiting BTK in HCL

B-cell receptor (BCR) signalling is important for the survival of HCL cells [206]. Molecules associated with the microenvironment like CXCR4, CCL3, CCL4, and CCL4 interact with the BCR and/or Bruton’s Tyrosine Kinase (BTK) and affect HCL survival [207,208]. The BTK inhibitor Ibrutinib is FDA approved for several B-cell malignancies including CLL and mantle cell lymphoma [209]. A case report of Ibrutinib treatment was noted in multiply relapsed HCLv [210]. Ibrutinib showed clinical benefit despite falling short of major response. A phase 2 multicenter trial of Ibrutinib (NCT01841723) was performed in 37 evaluable patients, 9 of whom were classified as HCLv and the rest as classic HCL [102]. Most patients began at 420 mg/day, and some of the 13 patients who started at 840 mg/day were dose-reduced to 420 due to toxicity. The primary endpoint was ORR which was 36% at 48 weeks, and 54% at best response. Responses improved with time, and eventually 7 (19%) achieved CR and 3 (8%) achieved MRD-free CR [102]. One of the HCLv patients with MRD-free CR had received HyperCVAD with rituximab and had positive cervical lymph nodes but negative blood and bone marrow. He was treated with 840 mg/day of Ibrutinib, and it took 2.5 years for lymph nodes to become negative by exam. Since the patient remained negative by bone marrow and blood, he was considered an MRD-free CR once the cervical lymph nodes became non-palpable. The median PFS was not reached and at 36 months the PFS was 73%. Toxicity from Ibrutinib is generally less than for BRAF/MEK inhibitors, although 43% had hypertension and 16% had atrial fibrillation. Hematologic toxicities included anemia (43%), thrombocytopenia (41%), and neutropenia (35%). Non-hematologic toxicities included diarrhea (59%), fatigue (54%), myalgias (54%) and nausea (51%). Ibrutinib was combined with venetoclax for a patient with both IGHV4-34−: HCLv and CLL [211], and Ibrutinib plus BR was used to treat a patient with HCL [212,213].

11. Treating HCL in the era of COVID-19

A review of 3377 patients showed that risk of death from COVID-19 was higher in patients with hematologic malignancies, particularly among patients hospitalized [214]. Clinical data on HCL patients infected with COVID-19 is limited. A case report describes a 54-year-old male with relapsed HCL 10 years after initial treatment who experienced respiratory failure from COVID-19 and had a difficult recovery [215]. A consensus document recommended caution in treating HCL patients with immunosuppressive agents during the COVID-19 pandemic, including both purine analogs which deplete T-cells and normal B-cells, and CD20 Mabs like rituximab which induce prolonged depletions of normal B-cells [216]. In addition to placing COVID-19 infected HCL patients at increased risk for adverse outcomes, immunosuppressive treatments pose a second potential problem, namely preventing effective vaccination. For this reason, the consensus document recommends that vaccinated HCL patients obtain antibody testing to confirm immunity [216]. It is known that rituximab prevents effective vaccination to influenza vaccine at least 6 months after the last dose [217], but data are not yet available for HCL patients. In the CLL population, no patients exposed to anti-CD20 Mab within 12 months responded to the Pfizer COVID-19 vaccine [218]. Even BTK-inhibitors were associated with impaired response to COVID-19 vaccination in CLL [219]. A study of 1445 patients with hematologic malignancies immunized with either the Moderna (mRNA-1273) or Pfizer (BNT162b2) vaccine included 650 patients with CLL and 7 with HCL [220]. All 7 HCL patients achieved positive levels of antibodies, compared with only 64.2% of the patients with CLL. The treatment status of the patients was not reported, but it is possible that HCL patients, particularly several years after first line purine analog therapy, have good immunogenicity to COVID-19 vaccines. In the algorithm of different standard and investigational treatments shown in Fig. 3, the only agents without known risk of causing prolonged immunosuppression or preventing effective immunization would
be Moxe, interferon and the BRAF and/or MEK inhibitors. The effect of Moxe on immunization should be minimal because of its short half-life [86,91,178]. In the phase 3 trial, Moxe decreased the number of B-cells much more transiently than would be expected from rituximab [91]. As mentioned above, it has been common during the pandemic for HCL patients to receive BRAF therapy even in 1st line as a bridge to more definitive cladribine or cladribine-rituximab treatment and initiate definitive therapy only after documentation of effective vaccination. It has been assumed but not proven that BRAF inhibition is not immunosuppressive, and that such treatment may be appropriate for newly diagnosed HCL patients who can’t achieve effective vaccination due to disease. How best to treat HCL patients who become infected with COVID-19 has not been reported. A recent cohort study reports 143 patients with hematologic malignancies who received convalescent plasma, with some suggestion of benefit [221]. Of these 143 patients, 123 (86%) had lymphoid malignancies, none reported as HCL. We anticipate clinical data to emerge soon regarding these important clinical issues, which remain relevant as the pandemic appears to continue.

Practice Points:

- Patients with HCL and HCLv require a proper diagnosis including flow cytometry of the blood and bone marrow, and molecular testing at least for absence of BRAF V600E when high-risk variants are suspected.
- Patients with HCLv should not receive purine analog monotherapy, even in 1st line.
- Up to 6 months may be required for HCL treatment to achieve complete remission. Therefore, the presence of residual disease and cytopneas within 4–6 months of treatment may not be an indication for retreatment; the cytopneas may be due to prior treatment and need more time to resolve.
- Cladribine with immediate or delayed rituximab is indicated in 2nd line treatment of HCL/HCLv, as it achieves MRD-free complete remission (CR) without chemotherapy toxicities. Adequate oral hydration during the first week of each cycle is important to prevent toxicities.
- Therapies targeted to BRAF, MEK and BTK have shown utility in treating relapsed HCL, and BRAF inhibition combined with CD20 Mab can achieve MRD-free CR.
- Prevention of effective immunization may occur due to HCL/HCLv comprising most or all B-cells or can occur from purine analogs, CD20 Mabs, or BTK inhibitors. Antibody testing is recommended in HCL to document effective vaccination, particularly to COVID-19, and particularly before beginning immunosuppressive therapies.

Research Agenda:

- Like vemurafenib-rituximab in relapsed HCL, vemurafenib-obinutuzumab can achieve MRD-free CR in 1st line, where it is being tested.
- Moxe combined with rituximab is under investigation to achieve MRD-free CR more quickly with lower risk of anti-drug antibodies (ADA). There is interest in testing this combination in 1st line.
- While BRAF inhibition cannot be used in HCLv, clinical trials are underway in HCLv testing Moxe-rituximab, the MEK inhibitor binimetinib, and CAR-T cell therapy targeting CD22.

Future considerations:

- To move the field of HCL/HCLv forward, more patients should be encouraged to participate in clinical trials. Many trials can be done without frequent travel. With more clinical data, treatment recommendations will become more evidence-based

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Declaration of competing interest

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