Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Blood Reviews 51 (2022) 100888

Contents lists available at ScienceDirect

Blood Reviews

journal homepage: www.elsevier.com/locate/issn/0268960X

Review

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

Robert J. Kreitman *, Evgeny Arons

Laboratory of Molecular Biology, Clinical Center, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

ARTICLE INFO

Keywords:
Hairy cell leukemia
Treatment
Cladribine
Rituximab
Vemurafenib
Dabrafenib
Trametinib
Moxetumomab pasudotox
Minimal residual disease

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 1st-line and relapsed settings. BRAF inhibitors and Ibrutinib 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

* Corresponding author at: National Institutes of Health, Building 37/5124b, 9000 Rockville Pike, Bethesda, MD 20892, USA.
E-mail address: kreitmar@mail.nih.gov (R.J. Kreitman).

https://doi.org/10.1016/j.blre.2021.100888

Available online 4 September 2021
0268-960X/Published by Elsevier Ltd.

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 1A (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 (MLL3), 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/leukemia, unclassifiable' [63], with SDRPL having less anemia, 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 HCLv [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, Maitre 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 $< 1–1.5 \times 10^9$ cells/L, hemoglobin <10–12 g/dL, and platelets $<100 \times 10^9$ 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 $>20 \times 10^9$/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 retreat 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 1.5×10^9, 11–12 g/dL, and 100×10^9/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 operator dependent, is insufficient. Rather, CT or MRI have to show >25% reduction from baseline or resolution to <17cm [91]. 2015 guidelines from ESMO require a chest X-ray and either ultrasound or CT to assess organomegaly and lymph nodes [88]. Resolution of lymph node size, not addressed in the consensus guidelines, requires reduction to <2cm in short axis [91].

5.2. CR with minimal residual disease (MRD)

With immunologic stains, HCL cells not detected by H/E can readily be quantified by immunohistochemistry (IHC). Flow cytometry of the blood and bone marrow using 2–8 antibodies at a time can detect as few

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; it would be inappropriate to withhold transfusion prior to treatment just to demonstrate 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 >2cm 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 × 10^9/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]. Neuropathy 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-naïve 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%) ($p < 0.0001$). 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

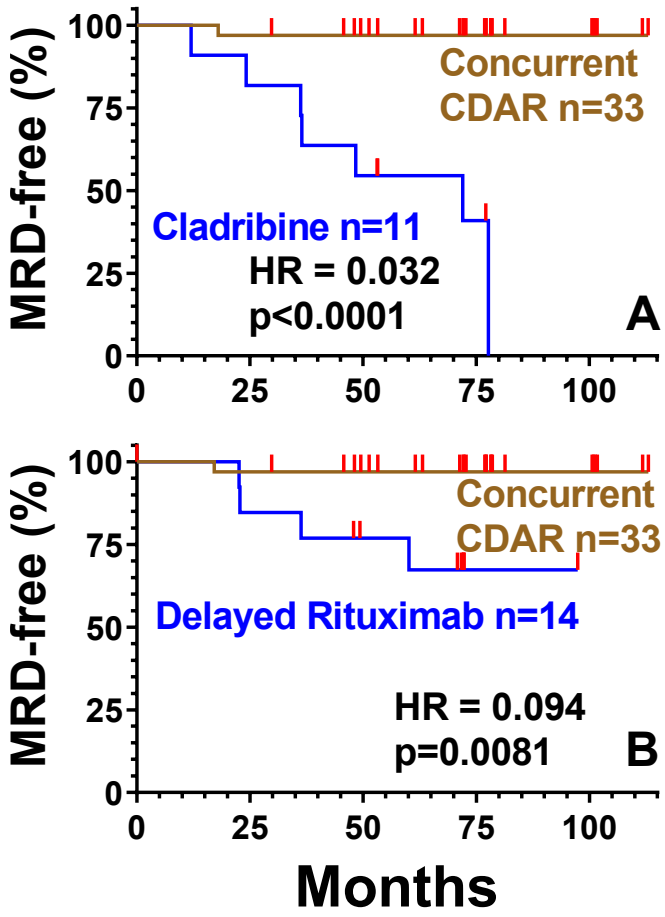

Fig 1. MRD-free survival in A of 33 patients after concurrent CDAR (brown) vs 11 patients after cladribine alone (blue). In B, MRD-free survival of the same 33 patients in A vs 14 patients achieving MRD-free CR after cladribine alone followed by delayed rituximab. Hazard ratios and p-values are shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

shows that of these 14 MRD-free patients after delayed rituximab, only 4 patients became MRD+ after delayed rituximab at a median of 6.5 years. We found that 32 (94%) vs 16 (47%) of patients ($p \leq 0.0001$) remained MRD-free after CDAR vs cladribine-delayed rituximab. Toxicity of CDAR in excess of cladribine was limited to transient thrombocytopenia, but CDAR was associated with higher neutrophil ($p = 0.017$) and platelet ($p = 0.0015$) counts at 4 weeks compared to cladribine monotherapy [87]. Compared to 28% of 90 historical patients who at 6.5 years relapsed from CR after purine analog with cytopenias requiring next therapy [21], only 1 (1.4%) of 68 patients needed next therapy by a median of 6.5 years after CDAR or delayed rituximab ($p < 0.0001$). Thus, not only MRD-free CR but also need for next treatment strongly favors CDAR or cladribine with delayed rituximab, compared with the standard of care approach of purine analog monotherapy without further therapy. The benefit of delayed rituximab is particularly relevant since so many thousands of patients have received cladribine alone and are MRD+ in blood. Thus, we believe that our data, combined with that of Ravandi et al, strongly argue that rituximab added to cladribine, either initially or delayed, be at least considered for 1st-line treatment of HCL. Some have recommended this combination approach for high-risk HCL [123], and CDAR is now recommend as an option for 1st line HCL treatment by the 2021 NCCN guidelines [124]. Results of CDAR suggest the potential utility of rituximab or other CD20 Mab combined with non-chemotherapy in 1st-line HCL treatment, and this will be discussed below.

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% [64,120]. 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.

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 Bendamustine-Rituximab	iv	HCL/ HCLv	≥2–3rd line	CD20	Yes	NIH
Moxetumomab pasudotox plus Rituximab	IV IV	HCL/ HCLv	≥2nd line	CD22	No	NIH
Chimeric Receptor T-cells (CAR-T)	IV	HCL/ HCLv	≥3rd line	CD22	Yes	NIH
Encorafenib-Binimetinib	Oral	HCL	≥2–3rd line	BRAF MEK	No	NIH
Binimetinib	Oral	HCL/ HCLv	≥2–3rd line	MEK	No	NIH
Vemurafenib With Obinutuzumab	Oral IV	HCL	1st line	BRAF & CD20	No	MSKCC
Vemurafenib-Cobimetinib And/or Obinutuzumab	Oral IV	HCL	≥3rd line	BRAF-MEK & CD20	No	Italy

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

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 immunoconjugates, 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%) of 21 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 hypoalbuminemia, 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.

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

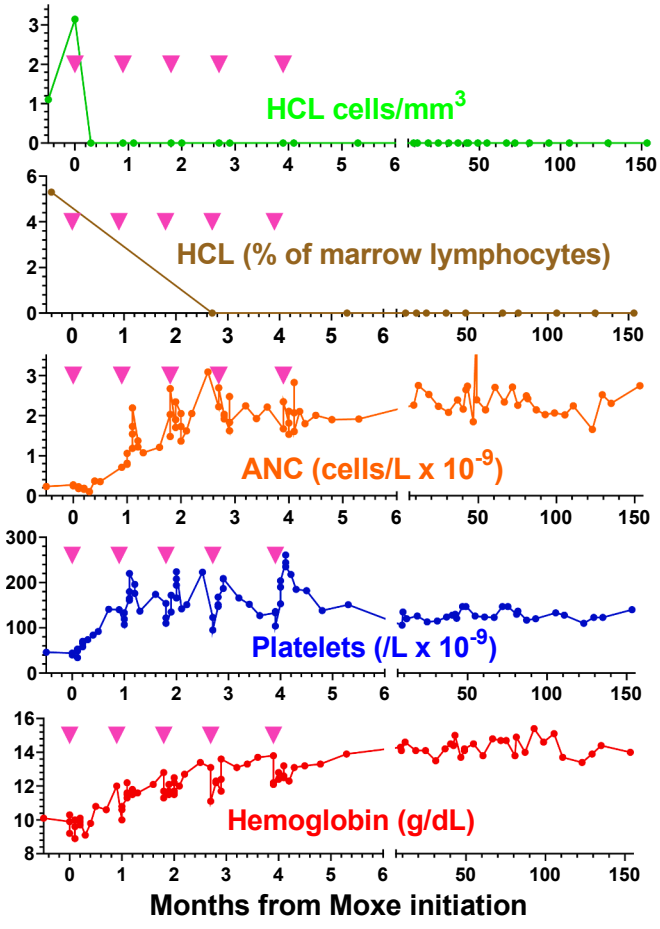

Fig. 2. MRD-free CR at 12.5 years after moxetumomab pasudotox (Moxe) initiation. The pink inverted triangles indicate the beginning of cycles of Moxe. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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 enrollment 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 cytopenia 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 cytopenias 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 Lumoxiti, 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 binging 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 hyponatremia 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.

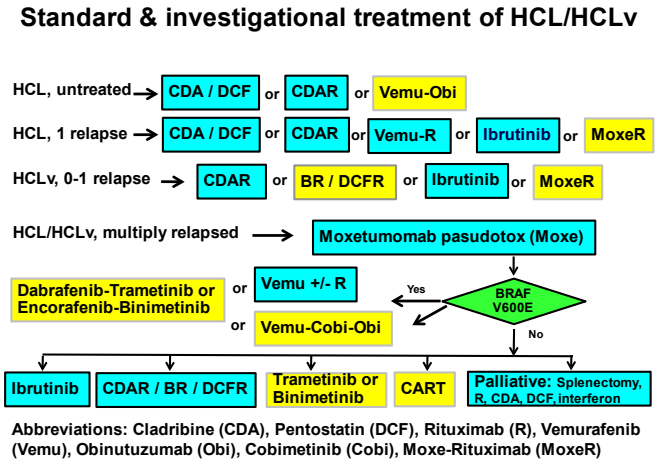

Fig. 3. Options which are FDA-approved or supported by published clinical trial data are blue surrounded by dark rectangles, while grey rectangles surround options in yellow which are currently under clinical testing or have been reported at meetings. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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 cytopenias [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 BMBx IHC [98]. In a retrospective trial, reduced dose vemurafenib at 240 mg/day was effective in HCL with resolved cytopenias in 21 patients [191]. At 480 mg/day, vemurafenib showed complete inhibition of signal-regulated kinase phosphorylation, and CR was achieved in 6 (40%) of 15 evaluable patients. Toxicities were still observed along with paradoxical ERK activation in BRAF WT cells [191]. Vemurafenib has been used as bridging therapy prior to definitive first-line therapy of HCL; due to its rapid reversal of cytopenias, 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

[195]. Dabrafenib monotherapy, in a single-center pilot phase 2 trial in 10 patents 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 cells 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 normal 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 cytopenias within 4–6 months of treatment may not be an indication for retreatment; the cytopenias 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 and is increasingly being used in 1st line to eliminate minimal residual disease (MRD) and prevent relapse.
- Moxetumomab pasudotox (Moxe) is FDA-approved for at least 3rd 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 immunosupressive 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

Funding

This work was supported by funding from the Intramural Research Program, National Institutes of Health, National Cancer Institute, Center for Cancer Research.

Declaration of competing interest

RJK is a coinventor on the NIH patent for Moxe. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Acknowledgements

The authors recognize the contributions of clinical and research staff of the Laboratory of Molecular Biology Clinical Immunotherapy Section. The following resources contributed to the work at NIH mentioned in the review: The NIH Intramural Program and research support from AstraZeneca, Novartis, Genentech, Teva, and Pfizer.

References

[1] Bouroncle BA, Wiseman BK, Doan CA. Leukemic reticuloendotheliosis. Blood 1958;13:609–30.
[2] Bouroncle BA. Leukemic reticuloendotheliosis (hairy cell leukemia). Blood 1979;53(3):412–36.
[3] Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. CA Cancer J Clin 2021;71(1):7–33.
[4] Tiacci E, Trifonov V, Schiavoni G, et al. BRAF mutations in hairy-cell leukemia. N Engl J Med 2011;364(24):2305–15.
[5] Tiacci E, Schiavoni G, Forconi F, et al. Simple genetic diagnosis of hairy cell leukemia by sensitive detection of the BRAF-V600E mutation. Blood 2012;119(1):192–5.
[6] Tiacci E, Pettirossi V, Schiavoni G, Falini B. Genomics of Hairy Cell Leukemia. J Clin Oncol 2017;35(9):1002–10.
[7] 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):3330–2.
[8] Green-Lott AM, Singaraju R, Liu ML, Ascensao J. Hairy cell leukemia and ground water contamination with industrial solvents: a case report. Mil Med 2020;185:e1338–40.
[9] Aristeguieta C, de Perio MA. Three cases of hairy cell leukemia in coal miners. Leuk Lymphoma 2011;52(12):2391–2.
[10] Hardell L, Eriksson M, Nordstrom M. Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: pooled analysis of two Swedish case-control studies. Leuk Lymphoma 2002;43(5):1043–9.
[11] Paydas S. Hairy cell leukemia and bladder cancer in a patient: relation with dye exposure and review of the literaure. Adv Hematol 2009;2009:812960.
[12] Tadmor T, Polliack A. Epidemiology and environmental risk in hairy cell leukemia. Best Pract Res Clin Haematol 2015;28(4):175–9.
[13] Golomb HM, Catovsky D, Golde DW. Hairy cell leukemia: a clinical review based on 71 cases. Ann Intern Med 1978;89(5 Pt 1):677–83.
[14] Quesada JR, Reuben J, Manning JT, Hersh EM, Gutterman JU. Alpha interferon for induction of remission in hairy-cell leukemia. N Engl J Med 1984;310(1):15–8.
[15] Spiers AS, Moore D, Cassileth PA, et al. Remissions in hairy-cell leukemia with pentostatin (2'-deoxycoformycin). N Engl J Med 1987;316(14):825–30.
[16] Johnston JB, Glazer RI, Pugh L, Israels LG. The treatment of hairy-cell leukaemia with 2'-deoxycoformycin. Br J Haematol 1986;63(3):525–34.
[17] 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):974–82.
[18] 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):1117–21.
[19] 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):1918–26.
[20] Robak T, Blasinska-Morawiec M, Blonski J, et al. 2-chlorodeoxyadenosine (cladribine) in the treatment of hairy cell leukemia and hairy cell leukemia variant: 7-year experience in Poland. Eur J Haematol 1999;62(1):49–56.
[21] 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;145:733–40.
[22] Goodman GR, Beutler E, Saven A. Cladribine in the treatment of hairy-cell leukaemia. Best Pract Res Clin Haematol 2003;16(1):101–16.
[23] Flinn IW, Kopecky KJ, Foucar MK, et al. Long-term results in hairy cell leukemia treated with pentostatin. Proc Am Soc Clin Oncol 1997;90(Suppl. 1):578a.

[24] Wierda WG, Byrd JC, Abramson JS, et al. Hairy cell leukemia, Version 2.2018, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 2017;15 (11):1414–27.
[25] 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):553–60.
[26] 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):2906–11.
[27] 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):42–7.
[28] Tadmor T. Purine analog toxicity in patients with hairy cell leukemia. Leuk Lymphoma 2011;52(Suppl. 2):38–42.
[29] Cheson BD, Vena DA, Foss FM, Sorensen JM. Neurotoxicity of purine analogs: a review. J Clin Oncol 1994;12(10):2216–28.
[30] 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):402–9.
[31] Hisada M, Chen BE, Jaffe ES, Travis LB. Second cancer incidence and cause-specific mortality among 3104 patients with hairy cell leukemia: a population-based study. J Natl Cancer Inst 2007;99(3):215–22.
[32] Paillassa J, Cornet E, Noel S, et al. Analysis of a cohort of 279 patients with hairy-cell leukemia (HCL): 10 years of follow-up. Blood Cancer J 2020;10(5):62.
[33] Quest GR, Johnston JB. Clinical features and diagnosis of hairy cell leukemia. Best Pract Res Clin Haematol 2015;28(4):180–92.
[34] Alsuliman T, Lassoued K, Belghoul M, Debbache K, Choufi B. Durable resolution of severe psoriasis in a patient treated with pentostatin for hairy cell leukemia: a case report. Dermatol Ther (Heidelb) 2018;8(1):165–9.
[35] Westbrook CA, Golde DW. Autoimmune disease in hairy-cell leukaemia: clinical syndromes and treatment. Br J Haematol 1985;61(2):349–56.
[36] Hasler P, Kistler H, Gerber H. Vasculitides in hairy cell leukemia. Semin Arthritis Rheum 1995;25(2):134–42.
[37] Rausch J, Windschmitt J, Schilling C, et al. Treatment-Induced aggravation of vasculitis in hairy-cell leukemia. Clin Lymphoma Myeloma Leuk 2021;21(5): e429–31.
[38] Robak E, Jesionek-Kupnicka D, Robak T. Skin changes in hairy cell leukemia. Ann Hematol 2021;100(3):615–25.
[39] Robak P, Jesionek-Kupnicka D, Kupnicki P, Polliack A, Robak T. Bone lesions in hairy cell leukemia: diagnosis and treatment. Eur J Haematol 2020;105(6): 682–91.
[40] Robak P, Jesionek-Kupnicka D, Kupnicki P, Polliack A, Robak T. Multifocal osteolytic lesions in hairy cell leukemia-the importance of PET/CT in diagnosis and assessment. Ann Hematol 2021;100:1641–5.
[41] Fasulo SM, Narvaneni S, Kumar V, Manje Gowda A, Sultana Y. Lytic bone lesion: an unusual presentation of hairy cell leukemia. Cureus 2021;13(1):e12959.
[42] Dedic K. Hairy cell leukemia: an autopsy study. Acta Medica (Hradec Kralove) 2003;46(4):175–7.
[43] Bigorra L, Larriba I, Gutierrez-Gallego R. The hairy cell leukaemia oxymoron: monocytotic monocytopenia. Clin Chem Lab Med 2020;59:e111–5.
[44] 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):401–9.
[45] Wotherspoon A, Attygalle A, Mendes LS. Bone marrow and splenic histology in hairy cell leukaemia. Best Pract Res Clin Haematol 2015;28(4):200–7.
[46] Sherman MJ, Hanson CA, Hoyer JD. An assessment of the usefulness of immunohistochemical stains in the diagnosis of hairy cell leukemia. Am J Clin Pathol 2011;136(3):390–9.
[47] Falini B, Tiacci E, Liso A, et al. Simple diagnostic assay for hairy cell leukaemia by immunocytochemical detection of annexin A1 (ANXA1). Lancet 2004;363(9424): 1869–70.
[48] 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.
[49] Gupta GK, Sun X, Yuan CM, Stetler-Stevenson M, Kreitman RJ, Maric I. The usefulness of novel dual color immunohistochemistry in detection of minimal hairy cell leukemia in bone marrow. Am J Clin Pathol 2019;153(3):322–7.
[50] 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.
[51] Salib C, Hussein S. A case of hairy cell leukemia with markedly hypocellular marrow mimicking aplastic anemia. Blood 2021;137(1):142.
[52] Durham BH, Getta B, Dietrich S, et al. Genomic analysis of hairy cell leukemia identifies novel recurrent genetic alterations. Blood 2017;130(14):1644–8.
[53] Maitre E, Bertrand P, Maingonnat C, et al. New generation sequencing of targeted genes in the classical and the variant form of hairy cell leukemia highlights mutations in epigenetic regulation genes. Oncotarget 2018;9(48):28866–76.
[54] 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.
[55] Dietrich S, Hullein J, Lee SC, et al. Recurrent CDKN1B (p27) mutations in hairy cell leukemia. Blood 2015;126(8):1005–8.
[56] Falini B, Martelli MP, Tiacci E. BRAF V600E mutation in hairy cell leukemia: from bench to bedside. Blood 2016;128(15):1918–27.
[57] Thompson ER, Lim KJC, Kuzich JA, et al. Detection of an IGH-BRAF fusion in a patient with BRAF Val600Glu negative hairy cell leukemia. Leuk Lymphoma 2020;61(8):2024–6.
[58] Arons E, Adams S, Pastan I, Kreitman RJ. Class II human leukocyte antigen DRB1*11 in hairy cell leukaemia patients with and without haemolytic uremic syndrome. Br J Haematol 2014;166(5):729–38.
[59] 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):547–59.
[60] Matutes E. Immunophenotyping and differential diagnosis of hairy cell leukemia. Hematol Oncol Clin North Am 2006;20(5):1051–63.
[61] Liu Q, Harris N, Epperla N, Andritsos LA. Current and Emerging Therapeutic Options for Hairy Cell Leukemia Variant. Onco Targets Ther 2021;14:1797–805.
[62] Matutes E. Diagnostic and therapeutic challenges in hairy cell leukemia-variant: where are we in 2021? Expert Rev Hematol 2021;14(4):355–63.
[63] Matutes E, Martinez-Trillos A, Campo E. Hairy cell leukaemia-variant: disease features and treatment. Best Pract Res Clin Haematol 2015;28(4):253–63.
[64] 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):3818–23.
[65] 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):6873–81.
[66] 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):487–95.
[67] 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):4687–95.
[68] 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): e57–60.
[69] Arons E, Kreitman RJ. Molecular variant of hairy cell leukemia with poor prognosis. Leuk Lymphoma 2011;52(Suppl. 2):99–102.
[70] Locher M, Jukic E, Bohn JP, et al. Clonal dynamics in a composite chronic lymphocytic leukemia and hairy cell leukemia-variant. Genes Chromosomes Cancer 2021;60(4):287–92.
[71] 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.
[72] Rahman ZA, Muwalla F, Jiang L, Foran J. Hairy cell leukemia with CCND1/IGH fusion gene and BRAF V600E mutation. Leuk Res Rep 2020;13:100197.
[73] Keruakous AR, Balakrishna P, Schmidt SA, Autry MT, Asch A. Genomic profiling is a supplemental diagnostic and therapeutic modality for hairy cell leukemia variant. Ann Hematol 2021;100:1629–30.
[74] Arons E, Zhou H, Sokolsky M, et al. Expression of the muscle-associated gene MYF6 in hairy cell leukemia. PLoS One 2020;15(2):e0227586.
[75] 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):3902–11.
[76] 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):467–72.
[77] Shackelford RE, Heldmann M, Eskandari F, et al. Marked retroperitoneal lymphadenopathy in hairy cell leukemia: a case report. Case Rep Oncol 2013;6 (3):493–6.
[78] 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):112–24.
[79] 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):e51–3.
[80] Tallman MS, Hakimian D, Rademaker AW, et al. Relapse of hairy cell leukemia after 2-chlorodeoxyadenosine: long-term follow-up of the Northwestern University experience. Blood 1996;88(6):1954–9.
[81] Kreitman RJ, Wilson WH, Robbins D, et al. Responses in refractory hairy cell leukemia to a recombinant immunotoxin. Blood 1999;94:3340–8.
[82] 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;18:1614–36.
[83] 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):241–7.
[84] 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):6719–29.
[85] 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):2983–90.
[86] 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):1822–8.
[87] Chihara D, Arons E, Stetler-Stevenson M, et al. Randomized phase II study of 1st-line cladribine with concurrent or delayed rituximab in patients with hairy cell leukemia. J Clin Oncol 2021;38:1527–38.
[88] Robak T, Matutes E, Catovsky D, Zinzani PL, Buske C, Committee EG. Hairy cell leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2015;26(Suppl. 5):v100–7.

[89] Consensus resolution: proposed criteria for evaluation of response to treatment in hairy cell leukemia. Leukemia 1987;1(4):405.
[90] 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):3007–15.
[91] Kreitman RJ, Dearden C, Zinzani PL, et al. Moxetumomab pasudotox in relapsed/refractory hairy cell leukemia. Leukemia 2018;32(8):1768–77.
[92] Salem DA, Scott D, McCoy CS, et al. Differential expression of CD43, CD81 and CD200 in classic versus variant hairy cell leukemia. Cytometry B Clin Cytom 2019;96(4):275–82.
[93] 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):6313–21.
[94] Kreitman RJ, Tallman MS, Robak T, et al. Minimal residual hairy cell leukemia eradication with moxetumomab pasudotox: phase 1 results and long-term follow-up. Blood 2018;131(21):2331–4.
[95] Tiacci E, De Carolis L, Simonetti E, et al. Vemurafenib plus rituximab in refractory or relapsed hairy-cell leukemia. N Engl J Med 2021;384(19):1810–23.
[96] 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):1665–70.
[97] 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):241–6.
[98] 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):1733–47.
[99] Kreitman RJ, Dearden C, Zinzani PL, et al. Moxetumomab pasudotox in heavily pre-treated patients with relapsed/refractory hairy cell leukemia (HCL): long-term follow-up from the pivotal Phase 3 trial. J Hematol Oncol 2021;14(1):35.
[100] Sarid N, Ahmad HN, Wotherspoon A, Dearden CE, Else M, Catovsky D. An unusual indication for splenectomy in hairy cell leukaemia: a report of three cases with persistent splenomegaly after chemoimmunotherapy. Br J Haematol 2015;171(5):784–7.
[101] Matsushita K, Margulies I, Onda M, Nagata S, Stetler-Stevenson M, Kreitman RJ. Soluble CD22 as a tumor marker for hairy cell leukemia. Blood 2008;112(6):2272–7.
[102] Rogers KA, Andritsos LA, Wei L, et al. Phase 2 Study of ibrutinib in classic and variant hairy cell leukemia. Blood 2021;137(25):3473–83.
[103] 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):2981–6.
[104] Goodman GR, Burian C, Koziol JA, Saven A. Extended follow-up of patients with hairy cell leukemia after treatment with cladribine. J Clin Oncol 2003;21(5):891–6.
[105] 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):217–29.
[106] Dong H, Shen Y, Shen Y, Wu D. Severe systemic rash in the treatment of hairy cell leukemia with cladribine: case report and literature review. Int J Gen Med 2020;13:1187–92.
[107] Castagna J, Amsler E, Kurihara F, Chasset F, Barbaud A, Soria A. Atypical features of cutaneous adverse drug reactions during therapy for hairy cell leukemia. J Allergy Clin Immunol Pract 2020;8(9):3203–6. e3201.
[108] Chacko J, Murphy C, Duggan C, O'Briain DS, Browne PV, McCann SR. Weekly intermittent 2-CdA is less toxic and equally efficacious when compared to continuous infusion in hairy cell leukaemia. Br J Haematol 1999;105(4):1145–6.
[109] Lauria F, Bocchia M, Marotta G, Raspadori D, Zinzani PL, Rondelli D. Weekly administration of 2-chlorodeoxyadenosine in patients with hairy- cell leukemia: a new treatment schedule effective and safer in preventing infectious complications [letter]. Blood 1997;89(5):1838–9.
[110] Lauria F, Bocchia M, Marotta G, Raspadori D, Zinzani PL, Rondelli D. Weekly administration of 2-chlorodeoxyadenosine in patients with hairy-cell leukemia is effective and reduces infectious complications. Haematologica 1999;84(1):22–5.
[111] Robak T, Jamroziak K, Gora-Tybor J, et al. Cladribine in a weekly versus daily schedule for untreated active hairy cell leukemia: final report from the Polish Adult Leukemia Group (PALG) of a prospective, randomized, multicenter trial. Blood. 2007;109(9):3672–5.
[112] Zenhausern R, Schmitz SF, Solenthaler M, et al. Randomized trial of daily versus weekly administration of 2-chlorodeoxyadenosine in patients with hairy cell leukemia: a multicenter phase III trial (SAKK 32/98). Leuk Lymphoma 2009;50(9):1501–11.
[113] Inbar M, Herishanu Y, Goldschmidt N, et al. Hairy cell leukemia: retrospective analysis of demographic data and outcome of 203 patients from 12 medical centers in Israel. Anticancer Res 2018;38(11):6423–9.
[114] Juliusson G, Heldal D, Hippe E, et al. Subcutaneous injections of 2-chlorodeoxyadenosine for symptomatic hairy cell leukemia. J Clin Oncol 1995;13(4):989–95.
[115] Sonderegger T, Betticher DC, Cerny T, Lauterburg BH. Pharmacokinetics of 2-chloro-2 '-deoxyadenosine administered subcutaneously or by continuous intravenous infusion. Cancer Chemother Pharmacol 2000;46(1):40–2.
[116] von Rohr A, Schmitz SF, Tichelli A, et al. Treatment of hairy cell leukemia with cladribine (2-chlorodeoxyadenosine) by subcutaneous bolus injection: a phase II study. Ann Oncol 2002;13(10):1641–9.
[117] Benz R, Arn K, Andres M, et al. Prospective long-term follow-up after first-line subcutaneous cladribine in hairy cell leukemia: a SAKK trial. Blood Adv 2020;4(15):3699–707.
[118] 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):177–83.
[119] Sigal DS, Sharpe R, Burian C, Saven A. Very long-term eradication of minimal residual disease in patients with hairy cell leukemia after a single course of cladribine. Blood 2010;115(10):1893–6.
[120] Chihara D, Kantarjian H, O'Brien S, et al. Long-term durable remission by cladribine followed by rituximab in patients with hairy cell leukaemia: update of a phase II trial. Br J Haematol 2016;174(5):760–6.
[121] Chow KU, Boehrer S, Geduldig K, et al. In vitro induction of apoptosis of neoplastic cells in low-grade non-Hodgkin's lymphomas using combinations of established cytotoxic drugs with bendamustine. Haematologica 2001;86(5):485–93.
[122] 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.
[123] Troussard X, Grever MR. The revised guidelines for the diagnosis and management of hairy cell leukaemia and the hairy cell leukaemia variant. Br J Haematol 2021;193(1):11–4.
[124] Wierda WG, Byrd JC, Abramson JS, et al. Hairy cell leukemia, Version 2.2021 NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 2021. https://www.nccn.org/guidelines/category_1.
[125] Angelova EA, Medeiros LJ, Wang W, et al. Clinicopathologic and molecular features in hairy cell leukemia-variant: single institutional experience. Mod Pathol 2018;31(11):1717–32.
[126] Matutes E, Wotherspoon A, Brito-Babapulle V, Catovsky D. The natural history and clinico-pathological features of the variant form of hairy cell leukemia. Leukemia 2001;15(1):184–6.
[127] Machii T, Chou T, Suzuki M, et al. Phase II clinical study of cladribine in the treatment of hairy cell leukemia. Int J Hematol 2005;82(3):230–5.
[128] Palomera L, Domingo JM, Sola C, Azaceta G, Calvo MT, Gutierrez M. Cladribine (2-chlorodeoxyadenosine) therapy in hairy cell leukemia variant. A report of three cases. Haematologica 2002;87(1):107–8.
[129] Tetreault SA, Robbins BA, Saven A. Treatment of hairy cell leukemia-variant with cladribine. Leuk Lymphoma 1999;35(3–4):347–54.
[130] Blasinska-Morawiec M, Robak T, Krykowski E, Hellmann A, Urbanska-Rys H. Hairy cell leukemia-variant treated with 2-chlorodeoxyadenosine – a report of three cases. Leuk Lymphoma 1997;25(3–4):381–5.
[131] Chihara D, Arons E, Stetler-Stevenson M, et al. Long term follow-up of a phase II study of cladribine with concurrent rituximab in patients with hairy cell leukemia variant. Blood Adv 2021. in press.
[132] Imoto N, Koyama D, Sugiura I, Kurahashi S. Long-term follow-up after rituximab plus bendamustine in a patient with relapsed or refractory hairy cell leukemia variant: a case report. Medicine (Baltimore) 2021;100(5):e24457.
[133] Gozzetti A, Sammartano V, Bacchiarri F, Raspadori D, Bocchia M. A BRAF-negative classic hairy cell leukemia patient with long-lasting complete remission after rituximab and pentostatin. Turk J Haematol 2020;37(4):286–7.
[134] Else M, Dearden CE, Matutes E, et al. Rituximab with pentostatin or cladribine: an effective combination treatment for hairy cell leukemia after disease recurrence. Leuk Lymphoma 2011;52(Suppl. 2):75–8.
[135] Hagberg H, Lundholm L. Rituximab, a chimaeric anti-CD20 monoclonal antibody, in the treatment of hairy cell leukaemia. Br J Haematol 2001;115(3):609–11.
[136] Lauria F, Lenoci M, Annino L, et al. Efficacy of anti-CD20 monoclonal antibodies (Mabthera) in patients with progressed hairy cell leukemia. Haematologica 2001;86(10):1046–50.
[137] 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):810–3.
[138] Thomas DA, O'Brien S, Bueso-Ramos C, et al. Rituximab in relapsed or refractory hairy cell leukemia. Blood 2003;102(12):3906–11.
[139] Angelopoulou MK, Pangalis GA, Sachanas S, et al. Outcome and toxicity in relapsed hairy cell leukemia patients treated with rituximab. Leuk Lymphoma 2008;49(9):1817–20.
[140] Zenhausern R, Simcock M, Gratwohl A, Hess U, Bargetzi M, Tobler A. Rituximab in patients with hairy cell leukemia relapsing after treatment with 2-chlorodeoxyadenosine (SAKK 31/98). Haematol Hematol J 2008;93(9):1426–8.
[141] Bohn JP, Gastl G, Steurer M. Long-term treatment of hairy cell leukemia with interferon-alpha: still a viable therapeutic option. Memo 2016;9:63–5.
[142] Kreitman RJ, Pastan I. Antibody fusion proteins: anti-CD22 recombinant immunotoxin moxetumomab pasudotox. Clin Cancer Res 2011;17(20):6398–405.
[143] Pastan I, Hassan R, FitzGerald DJP, Kreitman RJ. Immunotoxin therapy of cancer. Nat Rev Cancer 2006;6(7):559–65.
[144] Kreitman RJ, Pastan I. Immunotoxins in the treatment of refractory hairy cell leukemia. Hematol Oncol Clin North Am 2006;20(5):1137–51.
[145] Du X, Youle RJ, FitzGerald DJ, Pastan I. Pseudomonas exotoxin A-mediated apoptosis is Bak dependent and preceded by the degradation of Mcl-1. Mol Cell Biol 2010;30(14):3444–52.
[146] Messmann RA, Vitetta ES, Headlee D, et al. A phase I study of combination therapy with immunotoxins IgG-HD37- deglycosylated ricin A chain (dgA) and IgG-RFB4-dgA (Combotox) in patients with refractory CD19(+), CD22(+) B cell lymphoma. Clin Cancer Res 2000;6(4):1302–13.
[147] 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;82:2624–33.
[148] Kreitman RJ. Getting plant toxins to fuse. Leuk Res 1997;21:997–9.

[149] Du X, Beers R, FitzGerald DJ, Pastan I. Differential cellular internalization of anti-CD19 and-CD22 immunotoxins results in different cytotoxic activity. Cancer Res 2008;68(15):6300–5.
[150] Ogata M, Fryling CM, Pastan I, FitzGerald DJ. Cell-mediated cleavage of Pseudomonas exotoxin between Arg^{279} and Gly^{280} generates the enzymatically active fragment which translocates to the cytosol. J Biol Chem 1992;267:25396–401.
[151] Fryling C, Ogata M, FitzGerald D. Characterization of a cellular protease that cleaves *Pseudomonas* exotoxin. Infect Immun 1992;60:497–502.
[152] Chiron MF, Fryling CM, FitzGerald DJ. Cleavage of Pseudomonas exotoxin and diphtheria toxin by a furin-like enzyme prepared from beef liver. J Biol Chem 1994;269:18167–76.
[153] McKee ML, FitzGerald DJ. Reduction of furin-nicked Pseudomonas exotoxin A: An unfolding story. Biochemistry 1999;38(50):16507–13.
[154] Kreitman RJ, Pastan I. Importance of the glutamate residue of KDEL in increasing the cytotoxicity of Pseudomonas exotoxin derivatives and for increased binding to the KDEL receptor. Biochem J 1995;307:29–37.
[155] Theuer C, Kasturi S, Pastan I. Domain II of Pseudomonas exotoxin A arrests the transfer of translocating nascent chains into mammalian microsomes. Biochemistry 1994;33:5894–900.
[156] Theuer CP, Buchner J, FitzGerald D, Pastan I. The N-terminal region of the 37-kDa translocated fragment of *Pseudomonas* exotoxin A aborts translocation by promoting its own export after microsomal membrane insertion. Proc Natl Acad Sci U S A 1993;90:7774–8.
[157] Webb TR, Cross SH, McKie L, et al. Diphthamide modification of eEF2 requires a J-domain protein and is essential for normal development. J Cell Sci 2008;121(Pt 19):3140–5.
[158] Carroll SF, Collier RJ. Active site of *Pseudomonas aeruginosa* exotoxin A. Glutamic acid 553 is photolabeled by NAD and shows functional homology with glutamic acid 148 of diphtheria toxin. J Biol Chem 1987;262:8707–11.
[159] Brinkmann U, Brinkmann E, Gallo M, Pastan I. Cloning and characterization of a cellular apoptosis susceptibility gene, the human homologue to the yeast chromosome segregation gene CSE1. Proc Natl Acad Sci U S A 1995;92:10427–31.
[160] Keppler-Hafkemeyer A, Kreitman RJ, Pastan I. Apoptosis induced by immunotoxins used in the treatment of hematologic malignancies. Int J Cancer 2000;87(1):86–94.
[161] Decker T, Oelsner M, Kreitman RJ, et al. Induction of caspase-dependent programmed cell death in B-cell chronic lymphocytic leukemia cells by anti-CD22 immunotoxins. Blood 2004;103(7):2718–26.
[162] 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;339:394–7.
[163] Kreitman RJ, Batra JK, Seetharam S, Chaudhary VK, FitzGerald DJ, Pastan I. Single-chain immunotoxin fusions between anti-Tac and Pseudomonas exotoxin: relative importance of the two toxin disulfide bonds. Bioconjug Chem 1993;4:112–20.
[164] Kreitman RJ, Bailon P, Chaudhary VK, FitzGerald DJP, Pastan I. Recombinant immunotoxins containing anti-Tac(Fv) and derivatives of Pseudomonas exotoxin produce complete regression in mice of an interleukin-2 receptor-expressing human carcinoma. Blood 1994;83:426–34.
[165] Kreitman RJ, Chaudhary VK, Waldmann T, Willingham MC, FitzGerald DJ, Pastan I. The recombinant immunotoxin anti-Tac(Fv)-Pseudomonas exotoxin 40 is cytotoxic toward peripheral blood malignant cells from patients with adult T-cell leukemia. Proc Natl Acad Sci U S A 1990;87:8291–5.
[166] Kreitman RJ, Chaudhary VK, Waldmann TA, et al. Cytotoxic activities of recombinant immunotoxins composed of Pseudomonas toxin or diphtheria toxin toward lymphocytes from patients with adult T-cell leukemia. Leukemia 1993;7:553–62.
[167] Robbins DH, Margulies I, Stetler-Stevenson M, Kreitman RJ. Hairy cell leukemia, a B-cell neoplasm which is particularly sensitive to the cytotoxic effect of anti-Tac (Fv)-PE38 (LMB-2). Clin Cancer Res 2000;6(2):693–700.
[168] Kreitman RJ, Margulies I, Stetler-Stevenson M, Wang QC, FitzGerald DJP, Pastan I. Cytotoxic activity of disulfide-stabilized recombinant immunotoxin RFB4(dsFv)-PE38 (BL22) towards fresh malignant cells from patients with B-cell leukemias. Clin Cancer Res 2000;6:1476–87.
[169] 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;53:819–25.
[170] Theuer CP, Kreitman RJ, FitzGerald DJ, Pastan I. Immunotoxins made with a recombinant form of Pseudomonas exotoxin A that do not require proteolysis for activity. Cancer Res 1993;53:340–7.
[171] Mansfield E, Chiron MF, Amlot P, Pastan I, FitzGerald DJ. Recombinant RFB4 single-chain immunotoxin that is cytotoxic towards CD22-positive cells. Biochem Soc Trans 1997;25:709–14.
[172] Mansfield E, Amlot P, Pastan I, FitzGerald DJ. Recombinant RFB4 immunotoxins exhibit potent cytotoxic activity for CD22-bearing cells and tumors. Blood 1997;90:2020–6.
[173] 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;81:148–55.
[174] Moake JL. Thrombotic thrombocytopenic purpura and the hemolytic uremic syndrome. Arch Pathol Lab Med 2002;126(11):1430–3.
[175] Salvatore G, Beers R, Margulies I, Kreitman RJ, Pastan I. Improved Cytotoxic activity towards 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.
[176] Alderson RF, Kreitman RJ, Chen T, et al. CAT-8015: a second-generation pseudomonas exotoxin A-based immunotherapy targeting CD22-expressing hematologic malignancies. Clin Cancer Res 2009;15(3):832–9.
[177] Vainshtein I, Sun B, Roskos LK, Liang M. A novel approach to assess domain specificity of anti-drug antibodies to moxetumomab pasudotox, an immunotoxin with two functional domains. J Immunol Methods 2020;477:112688.
[178] Kuruvilla D, Chia YL, Balic K, et al. Population pharmacokinetics, efficacy, and safety of moxetumomab pasudotox in patients with relapsed or refractory hairy cell leukemia. Br J Clin Pharmacol 2020;86:1367–76.
[179] Feurtado J, Kreitman RJ. Moxetumomab Pasudotox: clinical experience in relapsed/refractory hairy cell leukemia. Clin J Oncol Nurs 2019;23(3):E52–9.
[180] Yurkiewicz IR, Coutre S, Ghesquieres H, Pastan I, Kreitman RJ. Moxetumomab pasudotox as re-treatment for heavily-pretreated relapsed hairy cell leukemia. Leuk Lymphoma 2021. in press.
[181] Hassan R, Williams-Gould J, Watson T, Pai-Scherf L, Pastan I. Pretreatment with rituximab does not inhibit the human immune response against the immunogenic protein LMB-1. Clin Cancer Res 2004;10(1):16–8.
[182] Pai LH, Wittes R, Setser A, Willingham MC, Pastan I. Treatment of advanced solid tumors with immunotoxin LMB-1: an antibody linked to *Pseudomonas* exotoxin. Nat Med 1996;2(3):350–3.
[183] Tiacci E, Carolis LD, Simonetti E, et al. The BRAF inhibitor vemurafenib plus rituximab produces a high rate of deep and durable responses in relapsed/refractory hairy cell leukemia: updated results of a phase-2 trial. Hematol Oncol 2019;37(Suppl. 2):110–1.
[184] Ullah R, Yin Q, Snell AH, Wan L. RAF-MEK-ERK pathway in cancer evolution and treatment. Semin Cancer Biol 2021. in press.
[185] 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):3911–20.
[186] 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):2610–1.
[187] Campochiaro C, Dagna L. BRAF mutations in erdheim-Chester disease. Dermatol Clin 2016;34(1):xi–xii.
[188] Kreitman RJ. Hairy cell leukemia-new genes, new targets. Curr Hematol Malig Rep 2013;8(3):184–95.
[189] Chapman PB, Hauschild A, Robert C, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 2011;364(26):2507–16.
[190] 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):2038–40.
[191] Dietrich S, Pircher A, Endris V, et al. BRAF inhibition in hairy cell leukemia with low-dose vemurafenib. Blood 2016;127(23):2847–55.
[192] Schlaweck S, Brossart P, Heine A. Vemurafenib as bridging therapy of hairy cell leukemia in a Jehovah's Witness patient. Ann Hematol 2021. in press.
[193] Smirnova SY, Al-Radi LS, Moiseeva TN, et al. Inhibitor of BRAF(V600E) mutation as a treatment option for hairy cell leukemia with deep neutropenia and infectious complications. Clin Lymphoma Myeloma Leuk 2021;21(7):427–30.
[194] Liebers N, Roider T, Bohn JP, et al. BRAF inhibitor treatment in classic hairy cell leukemia: a long-term follow-up study of patients treated outside clinical trials. Leukemia 2020;34(5):1454–7.
[195] 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. quiz 13.
[196] Tiacci E, De Carolis L, Simonetti E, et al. Safety and efficacy of the BRAF inhibitor dabrafenib in relapsed or refractory hairy cell leukemia: a pilot phase-2 clinical trial. Leukemia 2021. in press.
[197] Robert C, Karaszewska B, Schachter J, et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. N Engl J Med 2015;372(1):30–9.
[198] Subbiah V, Kreitman RJ, Wainberg ZA, et al. Dabrafenib and trametinib treatment in patients with locally advanced or metastatic BRAF V600-mutant anaplastic thyroid cancer. J Clin Oncol 2017;36(1):7–13.
[199] Dummer R, Ascierto PA, Gogas HJ, et al. Encorafenib plus binimetinib versus vemurafenib or encorafenib in patients with BRAF-mutant melanoma (COLUMBUS): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2018;19(5):603–15.
[200] Andritsos LA, Grieselhuber NR, Anghelina M, et al. Trametinib for the treatment of IGHV4-34, MAP2K1-mutant variant hairy cell leukemia. Leuk Lymphoma 2018;59(4):1008–11.
[201] Caeser R, Collord G, Yao WQ, et al. Targeting MEK in vemurafenib-resistant hairy cell leukemia. Leukemia 2019;33(2):541–5.
[202] Cervetti G, Galimberti S, Andreazzoli F, et al. Rituximab as treatment for minimal residual disease in hairy cell leukaemia. Eur J Haematol 2004;73(6):412–7.
[203] Cervetti G, Galimberti S, Andreazzoli F, et al. Rituximab as treatment for minimal residual disease in hairy cell leukaemia: extended follow-up. Brit J Haematol 2008;143(2):296–8.
[204] Ravandi F, Jorgensen JL, O'Brien SM, et al. Eradication of minimal residual disease in hairy cell leukemia. Blood 2006;107(12):4658–62.
[205] Robak T, Janus A, Jamroziak K, Tiacci E, Kreitman RJ. Vemurafenib and rituximab in patients with hairy cell leukemia previously treated with moxetumomab pasudotox. J Clin Med 2021;10(13). in press.
[206] Forconi F. Hairy cell leukaemia: biological and clinical overview from immunogenetic insights. Hematol Oncol 2011;29(2):55–66.

[207] Burger JA, Sivina M, Ravandi F. The microenvironment in hairy cell leukemia: pathways and potential therapeutic targets. Leuk Lymphoma 2011;52(Suppl. 2): 94–8.
[208] Sivina M, Kreitman RJ, Arons E, Buggy JJ, 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):177–88.
[209] Brown JR. Ibrutinib in CLL and B cell malignancies. Leuk Lymphoma 2014;55(2): 263–9.
[210] Bohn JP, Wanner D, Steurer M. Ibrutinib for relapsed refractory hairy cell leukemia variant. Leuk Lymphoma 2017;58(5):1224–6.
[211] Jain P, Kanagal-Shamanna R, Konoplev S, Zuo Z, Estrov Z. Biclonal IGHV-4-34 hairy cell leukemia variant and CLL - successful treatment with ibrutinib and venetoclax. Am J Hematol 2018;93(12):1568–9.
[212] Letendre P, Doll D. Novel therapeutics in the treatment of hairy cell leukemia variant. Leuk Res 2018;75:58–60.
[213] Visentin A, Imbergamo S, Frezzato F, et al. Bendamustine plus rituximab is an effective first-line treatment in hairy cell leukemia variant: a report of three cases. Oncotarget 2017;8(66):110727–31.
[214] Vijenthira A, Gong IY, Fox TA, et al. Outcomes of patients with hematologic malignancies and COVID-19: a systematic review and meta-analysis of 3377 patients. Blood 2020;136(25):2881–92.
[215] Bellmann-Weiler R, Burkert F, Schwaiger T, et al. Janus-faced course of COVID-19 infection in patients with hematological malignancies. Eur J Haematol 2020;105 (4):502–4.
[216] Grever M, Andritsos L, Banerji V, et al. Hairy Cell Leukemia and COVID-19. Adaptation of Treatment Guidelines Leukemia 2021;35(7):1864–72.
[217] Yri OE, Torfoss D, Hungnes O, et al. Rituximab blocks protective serologic response to influenza A (H1N1) 2009 vaccination in lymphoma patients during or within 6 months after treatment. Blood 2011;118(26):6769–71.
[218] Herishanu Y, Avivi I, Aharon A, et al. Efficacy of the BNT162b2 mRNA COVID-19 vaccine in patients with chronic lymphocytic leukemia. Blood 2021;137(23): 3165–73.
[219] Pleyer C, Ali MA, Cohen JI, et al. Effect of Bruton tyrosine kinase inhibitor on efficacy of adjuvanted recombinant hepatitis B and zoster vaccines. Blood 2021; 137(2):185–9.
[220] Greenberger LM, Saltzman LA, Senefeld JW, Johnson PW, DeGennaro LJ, Nichols GL. Antibody response to SARS-CoV-2 vaccines in patients with hematologic malignancies. Cancer Cell 2021;39(8):1031–3.
[221] Thompson MA, Henderson JP, Shah PK, et al. Association of convalescent plasma therapy with survival in patients with hematologic cancers and COVID-19. JAMA Oncol 2021;7(8):1167–75.