Orphan drugs for the treatment of hairy cell leukemia

Estella Matutes

EXPERT OPINION

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Introduction: Hairy cell leukemia (HCL) is a rare leukemia derived from mature B lymphocytes. Advances in the understanding of its pathogenesis have translated into the development of effective therapies. Interferon was the first agent that showed activity in HCL. However, most responses were partial and after stopping the drug, relapses occurred. The introduction of 2-deoxycoformycin (DCF) and 2-chlorodeoxyadenosine (CDA) represented a major progress in the management of HCL. Responses to these agents are seen in 95% of patients and > 75% are durable complete responses. However, 40% of patients relapse and although a second response may be achieved, this is of shorter duration. In such setting, strategies using Rituximab targeting CD20 combined with DCF or CDA and/or immunotoxins such as the anti-CD22 McAb linked to the truncated pseudomonas exotoxin have shown promising results in relapsed patients. The recent discovery of the presence of *BRAF* mutations in HCL has opened avenues for targeting this deregulated gene with *BRAF* inhibitors.

Areas covered: This manuscript summarises the disease features, pathogenesis, diagnosis and current and new targeted treatments for HCL. A literatura search on pub med has been undertaken and the most relevant references have been considered.

Expert opinion: HCL is a potentially curable disease with the available treatments. DCF or CDA should be the standard first line treatment. Patients with suboptimal responses to these drugs or multiple relapses should be treated with Rituximab plus DCF or CDA or immunotoxins.

1. Introduction

Hairy cell leukemia (HCL) is a distinct disease entity defined by its clinical, morphological, pathological, immunological and molecular features [1]. In the 1970s the therapeutic options for HCL were very limited as these patients were refractory to the available drugs used in other chronic lymphoid leukemias and lymphomas such as chronic lymphocytic leukemia (CLL) or follicular lymphoma. Splenectomy was the sole therapeutic approach that was partially effective in a subset of HCL patients in whom bone marrow infiltration was mild and cytopenias were mainly due to hypersplenism. Over the last three decades, a significant progress in the treatment of HCL has been made as a result of the introduction of drugs such the purine analogs 2´deoxycoformycin (DCF) and 2´chlorodeoxyadenosine (CDA) that have shown a remarkable activity in this disease. The use of DCF or CDA has contributed to change the natural history of HCL. Further to this, new targeted agents directed against cell surface antigens conjugated or not to toxins have emerged [2,3]. These treatments are commonly used in the small proportion of patients who are refractory to the purine analogs or who experience only partial responses (PR) or multiple relapses. An excellent review on the efficacy of the various treatments in HCL has been published by Grever and Lozanski [4]. Such progress has not been taken place in the

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variant form of HCL (HCL-variant), a disease that will not be discussed here since, although it shares some pathological features with HCL, it is at present considered biologically unrelated to the classical form [5-7].

The present therapies available for HCL, the potential use of other drugs and novel therapeutic avenues for this disease would be discussed here. In order to better understand the management of these patients, the use and efficacy of the drugs and development of targeted treatments, we will also briefly describe the clinical manifestations and pathogenic pathways underlying the development or progression of HCL.

2. Hairy cell leukemia

2.1 Clinical features

HCL is a clinicopathological entity with distinct clinical and pathological features. It was first reported by Ewald in 1923 but described as a pathological entity by Bertha Bouroncle in 1958 under the name of leukemic reticuloendotheliosis [8]. HCL occurs preferentially in middle age males with a male: female ratio of 5:1 and manifests with splenomegaly, pancytopenia and bone marrow infiltration. Cytopenias, particularly monocytopenia, are the most common manifestations but other features such as vasculitis, autoimmune hemolytic anemia or bone lytic lesions may be present in a minority of patients. The main sites of disease are the spleen and bone marrow, while peripheral lymphadenopathy is rare. Abdominal lymphadenopathy may be present in ~20% of patients, particularly during the evolution of the disease [9]. These patients tend to have some resistance to treatment with purine analogs [10] and therefore a whole body computerized tomography (CT scan) should be considered in the work-up to estimate the extent of the disease.

2.2 Laboratory features

The morphology, the immunophenotypic makeup of the neoplastic cells and the histology of the bone marrow and spleen are characteristic of this disease. Hairy cells are of medium size, with an oval or kidney-shaped rarely convoluted nucleus with a cotton–wool chromatin pattern and an abundant pale cytoplasm with irregular borders or hair-like projections (**Figure 1**). Immunophenotyping by flow cytometry shows that hairy cells are mature clonal B-lymphocytes that express a single immunoglobulin (Ig) light chain and that are preferentially IgG positive or usually express multiple Ig heavy chains. They express at a high density the antigens recognized by the monoclonal antibodies (McAb): CD19, CD20, CD22 and FMC7, while CD5 and CD23 are often negative. Expression of the antigens recognized by the McAb CD11c, CD25 against the Interleukin (IL)-2 receptor, CD103 and CD123 (anti-IL3 receptor) is characteristic of HCL (**Table 1**) [11,12].

Examination of the bone marrow trephine biopsy is essential for the diagnosis and assessment of response following therapy. The bone marrow often cannot be aspirated because of an increase in reticulin fibers (dry-tap bone marrow). If the bone marrow is aspirable, hairy cells may be seen admixed with hemopoietic precursors. In most patients, the bone marrow is hypercellular and the pattern of infiltration by hairy cells in the trephine biopsy is variable ranging from subtle to diffuse. The so-called fried-egg pattern, leaving clear spaces around the cells, is typical of this disease. In some cases, there is only a mild intrasinusoidal and/or interstitial bone marrow involvement that can be overlooked if immunohistochemical techniques are not carried out. Markers that are useful in tissue sections to detect hairy cells are Annexin A1, DBA44 and the McAb that detects tartrate-resistant acid phosphatase (TRAP). Of these, the most specific for lymphoid disorders is Annexin A1 [13]. Hairy cells in most cases weakly express cyclin D1 without evidence of the translocation t(11;14)(q13;q32) [14]. The spleen histology is characteristic of HCL with exclusive involvement of the red pulp and a naked white pulp. Pseudolake formation and extravasation of red cells are frequently seen.

A diagnostic workup on a patient with suspected HCL is shown in **Figure 2**.

2.3 Molecular genetics

HCL is not characterized by a recurrent chromosomal abnormality. Chromosomal abnormalities often involve the chromosome 5 with deletions or pericentric inversion, chromosome 7 and chromosome 14 at 14q32, the locus for the Ig heavy chain. High resolution genomic profiling in HCL, comparing it to HCL-variant, has demonstrated in both diseases a number of chromosome gains and losses, namely in chromosomes 5 and 7q [15]. However and in contrast to HCL-variant, losses at 17p – a region that codes for the tumor suppressor gene *TP53* or mutations of this gene – were not found in HCL and have rarely been reported in this disease [16]. The high frequency of *TP53* abnormalities in HCL-variant may well underline its refractoriness to agents that are very effective in HCL.

The putative cell of origin in HCL where the neoplastic transformation has probably taken place has been described in
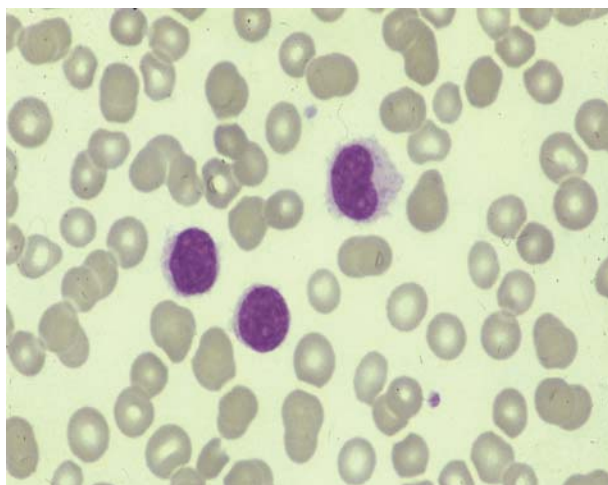
Figure 1. May Grunwald Giemsa stained peripheral blood film from a patient with HCL showing circulating hairy cells.

a number of studies and these suggested that transformation occurs in a post-germinal center memory cell. Most HCL cases have somatic mutations of the *IGHV* gene; unlike HCL-variant or splenic marginal zone lymphoma (SMZL), hairy cells lack specific *IGHV/IGHD/IGHJ* repertoires or stereotypes [17]. Microarray gene profiling has shown that HCL has a unique gene signature. *Cyclin D1*, *annexin A1*, *IL-3* receptor and *FLT-3* are upregulated and a variety of other genes are deregulated [18]. Although not yet explored, agents that target *FLT-3* could be potentially used in the treatment of refractory cases. A recent exciting finding is the presence of mutations on the *BRAFV600E* gene in virtually all HCL cases investigated, while mutations of this gene are very rare in other lymphoid disorders with only occasional case of SMZL, CLL or B-cell prolympho-cytic leukemia (B-PLL) being documented [19-22]. Only one study documented that a significant minority of HCL cases are wild type for the *BRAF* gene [23]. However, most of these cases had *IGHV4-34* rearrangement – a feature more characteristic of HCL-variant and not of HCL. The *BRAFV600E* mutation has been shown to be involved in the pathogenesis of various cancers such as melanoma and thyroid cancer and it appears to be the case in HCL. The mutation of the gene activates the Raf/MEK-ERK pathway providing an enhanced proliferation and longer survival to the cells. New and more feasible methods to be carried out on a routine basis detecting the mutation have been described such as quantitative real-time polymerase chain reaction (PCR) [22] and immu-nohistochemistry with a McAb that selectively detects the *BRAFV600E* gene when is mutated [21.] These latter techniques not only have a diagnostic potential but also may be useful to assess the quality of response following therapy by allowing to detect minimal residual disease (MRD). There are ongoing trials using inhibitors of the *BRAFV600E* gene in patients with *BRAF* mutated metastatic melanoma. In HCL this could also be a potential therapeutic target in patients who

have a suboptimal response or are refractory to purine analogs or immunotherapy.

2.4 Differential diagnosis (Table 1)

The differential diagnosis of HCL mainly arises with other splenomegalic lymphoid disorders and particularly with those with circulating villous lymphocytes such as SMZL and splenic B-cell lymphoma/leukemia unclassifiable (HCL-variant and splenic diffuse red pulp small B-cell lymphoma). Monocytopenia unlike in HCL is not a feature of these dis-eases that in addition have a morphology and immunopheno-type different from HCL. The pattern of bone marrow and spleen involvement in HCL is also different from that seen in SMZL although it overlaps with that of splenic B-cell lymphomas unclassifiable. Rarely the differential diagnosis of HCL arises with aplastic anemia in patients with a hypocel-lular bone marrow, myelofibrosis in those with subtle bone marrow involvement or mast cell disease.

2.5 Prognostic factors and outcome

The outcome of HCL patients has significantly changed due to the availability of effective therapies and better knowledge of the biology of this disease. This has resulted in median survivals of > 20 years compared to < 5 years in the 1980s. A number of clinical parameters shown to be predictors for a poor outcome are age, anemia or thrombocytopenia, spleno-megaly, shorter time from diagnosis to treatment, lower performance status and failure to achieve a complete response (CR) to purine analogs. [24-31]. However, it needs to be con-sidered that most of these predictive parameters were estab-lished in the past before effective treatments were available. Patients with bulky abdominal lymphadenopathy tend to have suboptimal responses to purine analog therapy [10]. One study has suggested that the uncommon HCL cases with *IgHV4-34* usage or with unmutated *IgVH* are those that more often do not respond to the purine analog CDA [16,32]. The very minority of HCL that have *TP53* abnormalities may also have an unfavorable outcome.

2.6 Treatment

2.6.1 Indications

Treatment is indicated in patients with symptomatic disease as manifested by cytopenias, bulky or progressive spleno-megaly, recurrent infections and/or systemic symptoms. A minority of patients is asymptomatic at diagnosis and a policy of watch and wait can be adopted – such is the case of asymptomatic stage A CLL patients.

The progress and landmarks of treatment in HCL are summarized in **Table 2**.
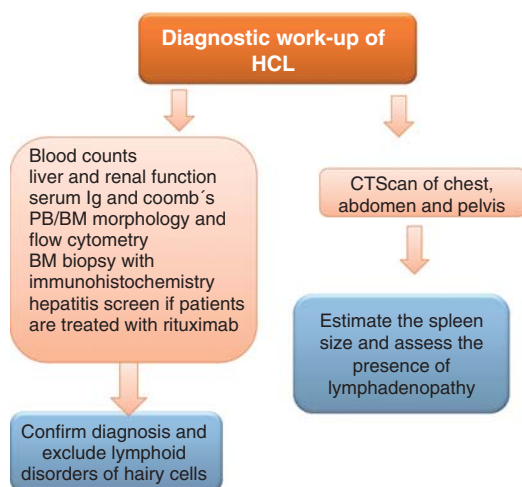
2.6.2 Splenectomy

In the past, splenectomy was the only effective therapeutic modality in HCL, particularly in those patients with bulky spleens and minor bone marrow involvement in whom cytopenias were mainly the result of hypersplenism. These

Table 1. Laboratory features of HCL and other splenomegalic B-cell disorders with villous lymphocytes.

	HCL	HCL-variant	DRSBCL	SMZL
Nucleus	Kidney shaped	Round	Round/oval	Round/oval
Nucleolus	No	Yes	No	No
Cytoplasm	Abundant	Abundant	Medium/small	Small
	Villous	Villous	Villous	Villous
			basophilia	basophilia
Spleen	Red pulp	Red pulp	Red pulp	marginal zone
	pseudolakes	pseudolakes +/-		
Bone marrow*	Interstitial/diffuse	Interstitial or diffuse	Interstitial	Interstitial
	spacing	spacing	nodular	nodular
CD11c	+++	+++	+/++	+/++
CD25	+++	neg	+	+
CD103	+++	++	+	neg
CD123	+++	neg	neg	neg
TRAP	+++	neg	NA	neg
Cyclin D1	+++	neg	NA	neg

*Intrasinusoidal infiltration may be present in all diseases.

+++: Positive in > 60% cases; ++: Positive in 30 – 60% cases; +: Positive in 10 – 30% of cases; neg: Positive in < 10% of cases; DRPSBCL: Diffuse red pulp splenic B-cell lymphoma; HCL: Hairy cell leukemia; HCL-v: Hairy cell leukemia variant; NA: Not available; SMZL: Splenic marginal zone lymphoma.

Figure 2. Diagnostic work up of a patient with HCL.

patients achieve hematological responses following splenectomy and although no complete remissions are observed, a small proportion may not require treatment for many years [33,34]. Splenectomized patients should receive vaccinations and prophylaxis with penicillin to prevent infections by encapsulated bacteria. Although at present there is no definitive role for splenectomy in HCL, patients with profound cytopenias and very bulky spleens might benefit from it prior purine analog therapy as it will facilitate the delivery of this drug.

2.6.3 Interferon-alpha
Quesada *et al.* first documented in 1984 the efficacy of Interferon-alpha (IFN-α) in HCL [35]. Out of the seven

patients treated with IFN-α, three achieved a CR and the remaining four achieved a PR. Subsequent studies in large cohorts of patients confirmed the activity of IFN-α in HCL [25,36-41]. The mechanism of action of IFN-α in HCL is not fully understood, may be multiple and in part by inducing the production of cytokines and growth factors. In most studies IFN-α has been administered at a dose of 2 or 3 × 10^6 IU subcutaneously (s.c.) three times a week for 12 to 18 months. Main side effects are flu-like symptoms, gastrointestinal symptoms and more rarely neurological symptoms including depression or memory loss. Myelosuppression during the first 3 months is common and there may be impairment of the liver function tests. Although the overall response rate (ORR) to IFN-α is high ranging from 75 to 90%, most responses are partial and only a minority of patients (5 – 30%) achieves a CR with still detectable hairy cells in the bone marrow. Furthermore, discontinuation of the therapy is almost always associated to recurrence of the disease within 6 to 25 months. Patients who undergo maintenance therapy may experience a longer remission but the disease ultimately will recur or will become resistant to IFN-α. A prospective randomized trial comparing DCF with IFN-α has shown the superiority of DCF over IFN-α [25]. The CR rate was 76% in patients treated with DCF versus 11% for patients treated with IFN-α. Therefore, this agent is no longer used as a first-line treatment in HCL considering the greater efficacy of purine analogs (see below) neither seems to add a benefit when used as a consolidation or maintenance in patients who achieved a response to DCF. Marotta *et al.* randomized 145 patients that had a response to DCF (CR or PR) to receive IFN-α or not. The results showed that there were no differences between the two arms regarding improvement of response or progression free of disease [42].

Table 2. Progress of treatments in HCL.

Agent	Ref.	Year
Splenectomy		1970s
Interferon-α	[35]	1984
2-deoxycoformycin	[43]	1984
2-chorodeoxyadenosine	[44]	1990
Immuntoxin conjugates	[73-83]	2000s

2.6.4 Purine analogs

The introduction of the two purine nucleoside analogs first DCF by Spiers *et al.* in 1984 [43] and subsequently CDA by Piro *et al.* in 1990 [44] represented a remarkable advance in the treatment of HCL and changed the natural history of HCL. Therefore at present, purine analog is considered the first-line treatment in HCL. A number of reports including large number of patients treated with one of these two purine analogs have substantiated the early findings as described below.

2.6.4.1 DCF

This is a purine analog that inhibits the enzyme adenosine deaminase (ADA) that is essential to the purine metabolism in lymphoid cells. Inhibition of ADA results on the accumulation of deoxyadenosine triphosphate metabolites that likely are responsible for the cytotoxicity in hairy cells.

A number of clinical studies have shown the efficacy of DCF in HCL. Different schedules for delivery of this drug have been used such as 4 mg/m^2/intravenously (i.v.) as a bolus every two or four weeks, 5 mg/m^2 on two consecutive days every two weeks, or 4 mg/m^2/weekly for 3 weeks and subsequent therapy every 8 weeks. The schedule of 4 mg/m^2/i.v. every two weeks is currently adopted in the routine practice since it is shown to be effective and has less side effects than other schedules, particularly opportunistic infections.

The ORR in patients with HCL either untreated or previously treated ranges from 79 to 100% with a CR rate of 33 – 93% (**Table 3**) [24,25,29,30,45-53]. The responses are long lasting and if relapse occurs, the patient may achieve a second response to DCF and/or responds to the other purine analog CDA. There is no evidence for a cross-resistance to these two drugs. Differences in the ORR and quality of responses (CR vs PR) in the different studies may well relate to the suboptimal drug delivery and/or disease burden.

2.6.4.2 CDA

CDA is a purine analog that, unlike DCF, is not an inhibitor of ADA. This drug is cytotoxic to lymphocytes as its accumulation in the cells creates a deoxynucleotide that induces DNA strand breaks and depletion of NAD and ATP.

Like DCF, a number of studies have demonstrated the efficacy of CDA in HCL [24,26,27,31,54-61]. Different routes and schedules of administration of CDA have been used. In the first study by Piro *et al.*, CDA was administered i.v. at a dose of 0.1 mg/kg/day for 7 days by continuous infusion as a single cycle. Other schedules employed have been 0.15 mg/kg i.v. weekly/ 6weeks, 3.4 mg/m^2/day s.c. per 7 days and 0.14 mg/Kg/day s.c. per 5 days. A prospective randomized study on 132 HCL patients who received CDA daily for 5 days versus once a week for 6 weeks have not shown differences in toxicity between the two arms [62]. However, data is not available on the duration of response using these two different schedules to deliver the drug.

The ORR to CDA in the different clinical studies is shown in **Table 4**. The ORR ranged from 75 to 100% and the CR rate from 75 to 98%. It is worth to note that patients that achieve a PR following one course of CDA may get a CR following a second course.

There have been no prospective randomized trials comparing DCF and CDA. This is likely due to the rarity of the disease and the fact that the two purine analogs are highly effective in HCL. However, from all data published it appears that there are no significant differences in terms of response duration, quality of response and ORR. Long-term follow-up studies have shown that responses to either drug are long lasting; however, it has also become apparent that there is no plateau for relapse-free survival and, in a long term, a substantial proportion of patients will relapse. Data for DCF shows an estimated disease-free survival at 10 years of 68.8 and 67% [28,29] and for CDA of 63 and 64% at a median follow-up of 9 and 9.7 years [27,63]. The study with a longer follow-up comes from Else *et al.* [24] and shows that at 15 years, 47 and 48% of patients treated with DCF and CDA, respectively, will relapse. Although a second or even a third response can be achieved with either purine analog, the proportion of patients achieving a second or third CR is less than when these drugs are used as first line; despite of this, there are no differences in the duration of response when used as a second or third time. Therefore in such patients with one or more relapses and, particularly in those in whom the response is not long-lived, there is a need for modern therapies aimed to cure the disease as described below (see Sections 2.6.5 and 2.6.6).

2.6.4.3 MRD and adverse events

Two issues that need to be considered are the following: i) the prognostic impact of residual hairy cells in the bone marrow following a response to the purine analog in terms of disease free survival and ii) the adverse events derived from this treatment.

Despite of the high CR rate with these two drugs, a proportion of HCL patients may have low level of residual disease detected either by immunohistochemistry, multicolor flow cytometry or molecular studies with consensus primers PCR [64-66]. There has been no consensus in the definition of MRD in patients with HCL. Large studies comparing the different methods to detect MRD and/or correlating with clinical outcome are lacking. The scenario of CR (MRD+) is different to that of PR in which residual leukemic cells are

Table 3. Responses to deoxycoformycin in HCL.

No of patients	Prior therapy*	ORR (%)	CR (%)	PR (%)	Ref.
15	15	93	93	0	[45]
23	13	91	87	4	[46]
28	18	100	89	11	[47]
33	30	78	33	45	[48]
49	31	96	44	52	[49]
50	31	84	64	20	[50]
66	66	79	56	23	[51]
80	43	98	72	16	[30]
85	85	84	42	42	[52]
148	23	96	74	22	[53]
154	0	79	76	3	[25]
188[‡]	108	96	82	14	[24]
238	154	95.6	79	16.6	[28]
240	86	72	NA	NA	[29]

*Including splenectomy and/or Interferon and/or others.
[‡]Patients in this series were also included in reference 53.
CR: Complete response; ORR: Overall response rate; PR: Partial response.

Table 4. Responses to CDA in HCL.

No of patients	Prior therapy*	ORR (%)	CR (%)	PR (%)	Ref.
97	41	95.9	77.3	18.6	[54]
29	14	96	86	10	[55]
37	12	100	78	22	[56]
46	27	89	78	11	[57]
44	11	98	98	0	[58]
48	18	100	76	24	[24]
49	28	100	76	24	[59]
50	15	100	80	20	[60]
144	75	97	85	12	[61]
196	na	100	95	5	[27]
349	176	98	91	7	[31]
861	513	87	50	37	[26]

*Including splenectomy and/or Interferon and/or others.
CR: Complete response; ORR: Overall response rate; PR: Partial response.

easily detected by conventional methods. A PR achievement predicts for a less durable response compared to CR [24] and therefore it is important to continue with further therapy until a maximal response is obtained. In contrast, there are uncertainties concerning the clinical significance of the presence of MRD in HCL and whether its detection would predict for an early relapse. This has not been evaluated in prospective randomized studies including a large number of patients with a long follow-up and therefore results need to be taken with caution.

The two purine analogs result in a profound immunosuppression with lymphopenia and particularly affecting the CD4^{+} T-lymphocytes. Opportunistic infections and particularly reactivation of the varicella zoster virus may be seen. Although there has not been a study showing that antiviral prophylaxis with acyclovir and pneumocystis carinii prophylaxis (PCP) with cotrimoxazole may prevent these infections,

it will be advisable to administer cotrimoxazole and acyclovir to all HCL patients during the treatment with the purine analog and 6 to 12 months after stopping therapy. Other potential side effects, which are very uncommon with these drugs, are renal failure and hypoplasia of the bone marrow that usually recovers in a long term.

2.6.5 Purine analog plus antibody therapy

These schedules include targeted therapies directed against cell surface antigens. Rituximab targeting the CD20 antigen has been used as a single agent in a few studies [67,68]. However, results with Rituximab as single agent are less impressive that those obtained with single purine analog agent. In contrast to the purine analogs, responses to Rituximab are achieved in 30 – 50% of patients and less than 20% are CR. By contrast the combination of chemoimmunotherapy (Rituximab plus DCF or CDA) in patients who experience
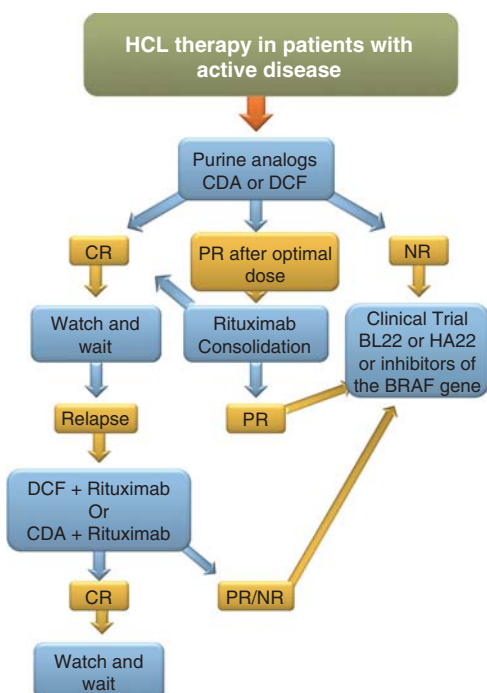
Figure 3. Flow chart illustrating the treatment pathways in HCL.

early relapse, multiple relapses or do not achieve a response to single agent purine analog demonstrates that this combination is highly effective [69,70]. Else *et al.* reported the efficacy of a combination of DCF or CDA with Rituximab in eight multiple relapsed or refractory HCL patients with impressive results [69]. An update of this study in 18 patients that had previously received one to six therapies has shown an ORR of 100%, a CR rate of 89% and that 16 of 18 patients treated with this combination remain in CR with a median follow-up of 36 months; one PR patient relapsed at 10 months [71]. Therefore the vast majority of these patients achieve a long-lasting CR to this combination. However there are uncertainties as to whether which is the best schedule to deliver these drugs (combined or sequential) and whether this combination is superior to single purine analog when used as first line.

Single agent Fludarabine, another purine analog that has shown to have a high activity in CLL, has been rarely used in HCL given the efficacy of the two other purine analogs. However, a recent study by Gerrie *et al.* [72] in 15 HCL patients with refractory/relapsed disease using a combination of Fludarabine and Rituximab has shown an ORR of 100% and, at a median follow-up of 35 months, 14 of the 15 patients maintain the response. The 5-year progression-free survival and overall survival are 83 and 89%.

2.6.6 Immunochemotherapy and immunotoxins

New therapeutic strategies, essentially recombinant immuno-toxins conjugates have been developed for HCL patients with truly refractory disease. These are fusion proteins that link variable fragment domains (Fv) of an antibody directed to a B-cell antigen strongly expressed by hairy cells with the toxic portion of a toxin. The Fv of the antibody binds to the antigen in the cell surface and delivers the toxin into the cytosol where it exerts cytolytic effect. [73-76]. Since 1999 several immunotoxins have been used in Phase I and Phase II trials in HCL and most if not all studies come from the National Cancer Institute in Bethesda (USA). One of the first drugs developed was the compound LMB-2 that fuses the Fv of the McAb anti-CD25 (anti-IL-2 receptor) with a 38KD truncated pseudomonas exotoxin. Although the anti-CD25 McAb does not internalize when delivered by its own, it does when conjugated to an exotoxin. A Phase I trial in four patients with resistant HCL showed responses in all of them including a long-lasting CR. [77]. Another agent designated BL22 that is an immunotoxin conjugate that delivers the pseudomonas toxin into cells expressing CD22 was later developed. A Phase I trial using this agent at a dose of 3 to 50 µg/kg/ every other day × 3doses in 31 patients with refractory HCL showed a CR rate of 61% increasing to 86% when a higher dose was used and a PR rate of 19% [78]. The median duration of response was 36 months. Most common side effects were hypoalbuminemia, fatigue, edema and, in four patients, a reversible hemolytic uremic syndrome that required dialysis. Neutralizing antibodies occurred in 24% of patients A subsequent Phase II trial with this drug in 36 patients who had a short response duration (less than 12 months) or lasting for 1 – 4 years to CDA and/or were refractory to this purine analog showed a CR rate of 47% and a PR rate of 25% after two cycles of treatment. The ORR (CR and PR) was higher in patients who had non-bulky splenomegaly (spleens < 14 cm) compared to those with bulky spleens (> 20 cm) or those who did not have splenomegaly [79]. A modified drug mutant of BL22, with mutations in the heavy chain complementary determinant region 3 (CDR3) designated moxetumomab pasudotox (HA-22), has been produced and shown to have an increased cytotoxic activity [80,81]. A Phase I dose–escalation trial to assess the safety and response to this drug in 28 HCL patients who had multiple relapses has been conducted. The patients had two or more previous lines of chemotherapy. The compound was delivered at doses of 5, 10, 30, 40 and 50 µg/kg q.o.d. × 3 for 1 to 16 cycles. The median of cycles received was 4. The ORR was 83% and seen at all doses and the CR rate was 46%. [82,83]. The latter were long-lasting with only one patient having relapsed within one year. Further, the profile was safe at all doses. Grades 1 – 2 hypoalbuminemia, edema, nausea and fatigue were documented in 25 – 64% of patients. Two patients had also grade 2 uremic hemolytic syndrome. Therefore moxetumomab pasudotox administered at a dose of 50 µg/kg/q.o.d. × 3 has activity in refractory HCL with a safe profile [83].

New therapeutic avenues need to be developed for HCL. Thus, despite of the exquisite sensitivity of HCL to the

available purine analog drugs, most of the patients will experience a relapse, and repeated courses of purine analogs might carry the risk of developing myelodysplasia or solid tumors. Drugs targeting the bone marrow microenvironment which may be the source of residual and/or refractory disease or those directed against genes that are deregulated in HCL, such as *FLT-3* and *BRAF*, need to be considered in the therapeutic scenario. The rationale for employing a *BRAF* inhibitor has been established by Tiacci *et al.* [19] that showed that *in vitro* treatment of hairy cells with a *BRAF* inhibitor causes dephosphorilation of MEK and ERK, thereby turning off the oncogenic Raf-MEK-ERK pathway. Recently, a HCL patient refractory to purine analog therapy responded to vemurafenib, a *BRAFV600E* inhibitor reinforcing that HCL is likely a kinase-dependent disease that can be treated with a targeted drug [84].

3. Expert opinion

HCL is a rare distinct and well-defined clinicopathological entity for which very effective treatments have become available for the last three decades. Hence, the outcome and survival of these patients has dramatically changed from a median survival of < 5 years in the 1970s to > 20 years at present. Indeed the life expectancy of a patient with HCL is not different to the general population. Two issues that need to be considered when first dealing with a patient with HCL are the following: i) Do all patients require treatment at diagnosis? ii) Which is the best therapeutic option and how we need to assess response to the treatment? With regards to the first question, treatment may well be postponed in a small proportion of patients without cytopenias and who do not have or have mild splenomegaly. In my experience, some of these patients may never require treatment in their life span. Treatments are not harmless and therefore a policy of watch and wait with close monitoring of the blood counts is reasonable. Considering the second question, it is clear that the purine analogs DCF or CDA are the gold standard first-line treatment for this disease. The fact that there have been not randomized clinical trials comparing these two drugs makes difficult the choice of one or another as initial therapy. The response rates and quality and duration of responses appear to be similar although the form of delivery is different. DCF is given sequentially i.v. every 2 weeks over a period of months and has the advantage of the possibility of a more closer monitoring in terms of delaying or reducing a dose and/or giving growth factors if the patient experiences profound cytopenias (neutropenia) due to drug toxicity rather than disease activity. CDA is usually given i.v. or s.c. as a block during 5 or 7 days. The advantages are the easier delivery via the s.c. form and that only needs one or perhaps two courses. Since the efficacy of these two drugs is similar, all these practical issues should be discussed individually with the patient. Prophylaxis with acyclovir and cotrimoxazole is recommended all through the treatment and up to 6 – 12 months after stopping the drugs. It is also important to assess the response to the purine analogs adequately. A bone marrow trephine biopsy with immunohistochemistry following treatment is mandatory regardless a complete resolution of the cytopenias is achieved. This will allow assessing the quality of response and, if a significant disease is left (i.e., PR), it is important to pursue with the treatment in order to achieve the best quality of response (CR). A practical aspect is that the bone marrow assessment should be carried out 3 or 4 months after therapy since the purine analogs work along several months. Another issue is how we manage patients that despite an optimal treatment with the purine analog only achieve a PR, those that relapse and/ or have truly refractory disease. There are various alternatives to approach these patients, as the scenarios are different. Patients who achieve a PR following the optimal doses of a purine analog might well benefit from Rituximab as a consolidation without increasing toxicity and resulting in the conversion of the PR to a CR. Although there have not been studies in a large cohort of these patients, it seems that this approach is safe and feasible. Patients who had achieved a durable response and of good quality to a purine analog and relapse, may well be retreated with the same or the alternative purine analog. However, if the initial response has been of short duration, < 2 years, they should be treated with a combination of a purine analog and Rituximab. There is evidence albeit in small series of patients that this combination results in a very high and durable CR rate. The minority of patients who are purine analog refractory should be enrolled, when possible, on clinical trials that use immunotoxin conjugates. Hopefully in a near future we will be able to use new agents targeting the mutated *BRAF* gene in refractory patients and it will not be surprising that clinical trials are set up to see whether this agent or immunotoxin conjugates when used as first line are even superior to the current standard therapies in HCL.

A flow chart on the management and therapeutic options in HCL is illustrated in **Figure 3**.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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