The treatment of hairy cell leukemia with a focus on long lasting responses to cladribine: A 30-year experience

Research Article

The treatment of hairy cell leukemia with a focus on long lasting responses to cladribine: a thirty-year experience.

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Abstract

The treatment of hairy cell leukemia (HCL) has considerably changed over years. Purine analogues, namely cladribine, now represent the treatment of choice.

One hundred and eighty-four patients were followed between 1986 and 2018 and treated according to era-specific guidelines. Responses were classified by combining Consensus Resolution criteria and marrow immunohistochemistry. Patients were grouped according to the number of treatment lines they received.

Patients treated first line responded in 86% of cases, with complete response (CR) in 44% of cases. Response rates remained high throughout the first 4 lines (84%, 81%, 79% for the second line onward, with CR in 38%, 37%, 15% of cases respectively). One hundred and twenty-two patients received cladribine as first line treatment, with a response rate of 86% and a CR rate of 54%. Among the 66 CR patients, 45 (68%) have never received further therapy: 11 patients are in continuous CR between 5 and 10 years after treatment, 14 between 10 and 20 years and 3 patients at more than 20 years. Median time-to-next treatment (TTNT) for frontline cladribine-treated patients was 8.2 years: partial responders had a significantly shorter median TTNT than CR patients (5.3 years versus median not reached at 25.8 years, p=0.0001).

Patients with HCL require subsequent lines of therapy in more than 50% of cases. Purine analogues allow significant response rates when applied first line and upon retreatment. Some patients may enjoy long lasting treatment-free intervals after one course of cladribine.

Key words. Cladribine, hairy cell leukemia, moxetumomab pasudotox, purine analogues, time-to-next treatment.

Background

Since the first description of leukemic reticuloendotheliosis in 1958,[1] only later defined as hairy cell leukemia (HCL),[2] no specific treatment able to modify the natural history of the disease has been made available for at least twenty years. Splenectomy was regarded as the only affordable approach, with merely palliative intentions, aimed at reducing the abdominal discomfort caused by spleen enlargement and at ameliorating peripheral blood cytopenias, although transiently.[3,4]

The first disease-modifying drug was interferon-alpha, introduced in the early eighties of the last century: its use was able to induce objective responses in HCL patients, as it appeared capable to resolve cytopenias and to reduce bone marrow leukemic cells, up to their possible complete clearance.[5]

High overall and complete response (CR) rates have been later described with purine analogues, namely pentostatin (or 2'-deoxycoformycin) and cladribine (or 2-chlorodeoxyadenosine), both available since the late eighties and the beginning of the nineties.[6,7] Besides their activity on the disease, purine analogues could induce long lasting responses following just one induction course.[8-11]

More recently, a deep awareness of the antigenic phenotype of the leukemic cell, along with new discoveries about the molecular pathogenesis of the disease,[12] have driven the development of new targeted drugs, to be applied in the context of disease relapse or refractoriness, when purine analogues do not constitute an option. Moxetumomab pasudotox has been developed as an immunotoxin targeting the surface CD22 antigen, and it is now approved as a third-line option in multiply relapsing HCL patients. This drug was able to induce a durable CR in 30% of the treated patients in the registration trial, with a meaningful clinical response in three quarters of them.[13] On the other hand, given the importance of the BRAF V600E mutation in the pathogenesis of the disease, which

determines the constitutive activation of the mitogen-activated protein kinases pathway proliferating signal, light was shed on the role of BRAF inhibitors in the treatment of the disease.[12] Vemurafenib, a small BRAF blocker given orally, produced clinically meaningful responses in 96.1% of treated patients according to a pivotal Italian trial, with CR in 34.6% of the cases.[14] All responses occurred rather early during the course of the treatment, confirming the role of the BRAF-mutated interference with the leukemogenic process. [14] The combination of vemurafenib and rituximab, indeed, has provided very good CR rates in heavily pretreated patients in a recently published phase 2 academic trial, thus emerging as a new possible standard of treatment in multiply relapsing cases.[15]

The history of the treatment of HCL is instructive, as the continuous discovery of new molecules and their application in possible future combinations have permitted to change the fate of an almost incurable disease into a highly controllable one, with survival rates roughly approximating those of the healthy population.[16] The recent coronavirus disease 2019 (COVID-19) pandemic has posed new treatment challenges, especially at disease onset. Due the highly immunosuppressive effect of purine analogues that may induce an increased susceptibility to a more severe course of COVID-19 infection, it has been recommended to establish an active surveillance in patients with low but stable blood counts, while considering the use of targeted agents – albeit applied “off-label” – as first-line options, given their reduced immunosuppressive potential.[17]

This works presents our institutional experience with the treatment of HCL patients over a period of time of thirty years, which covers all the approaches applied for the treatment of this disease, ranging from splenectomy, to purine analogues and newer targeted agents.

Patients and Methods

Aim of the study was to describe the era-specific treatments for patients with classic HCL during a period of at least thirty years, by evaluating remission rates and relapse rates for each treatment line applied either as induction or in case of relapse. The main study objective was to describe a population treated with frontline cladribine, pointing out that a proportion of patients may result treatment-free for a considerable amount of time after having received only one cladribine course. The primary endpoint of the study was therefore the time-to-next treatment (TTNT) interval. Secondary endpoints were represented by overall survival (OS) and overall response rate (ORR), defined as the sum of CR, partial and minor responses, as defined below in this paragraph.

For these purposes, a retrospective study was conducted, reviewing our single-centre database and all consecutive patients with a diagnosis of classic HCL in need of treatment have been included in the analysis.[18] Diagnosis was confirmed by bone marrow biopsy, which always included CD20 assessment by immunohistochemistry.

Treatment choice was based on era-specific guidelines.[16,18] Interferon-alpha and splenectomy were mainly applied before the advent of purine analogues. Cladribine was available at our institution since 1991. It was administered both intravenously and subcutaneously (0.14 mg/kg), for 5-7 consecutive days or once a week for 5 consecutive weeks.[19,20] Single-agent rituximab (375 mg/m^2) was considered suitable in patients with relapsed disease, deemed ineligible for retreatment with purine analogues.[21,22] New molecules, such as vemurafenib (starting dose of 960 mg twice daily) or moxetumomab pasudotox (40 μg/kg on days 1, 3, 5 every 28 days up to 6 cycles), have been applied as advanced lines in heavily pre-treated patients, as soon as they became available within clinical trials (beyond 2010).

Responses have been categorized according to the Consensus Resolution Criteria published in 1987.[23] Importantly, we applied an immunohistochemistry threshold for CD20 in order to refine the category of CR. Patients were stringently considered as complete responders if they met both the Consensus Resolution criteria for a CR and displayed a bone marrow CD20 positivity less than (or equal to) 10% of marrow cellularity, in absence of any residual infiltrate appreciated with hematoxylin-eosin staining.

Safety and tolerability were evaluated by recording incidence, severity, and type of any adverse event (AE) according to the National Cancer Institute Common Terminology Criteria for AEs v4.0.

The study was approved by our institutional board and by our ethical committee and performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its amendments. Patients were consecutively enrolled to avoid selection bias.

Demographics and patients' characteristics as well AEs were summarized by descriptive statistics. Continuous variables were reported as median (range) for non-normally distributed data and categorical variables as absolute and relative frequencies. All the time-to-event endpoints included in the study have been estimated with the Kaplan-Meier method. All analyses were performed using STATA (version 11.1; College Station, TX, USA).

Results

One hundred and eighty-four patients have been identified between 1986 and the end of 2018. As expected, more than 83% of patients were males. Median age at diagnosis was 56 (range: 27-84) years. Neutropenia and thrombocytopenia were the most frequent indications for treatment, and a clinically meaningful splenomegaly was detected in more than half of patients at disease onset. A detailed breakdown of patient's clinical characteristics and hematological parameters at disease onset is reported in Supplementary Table 1.

Purine analogues (namely cladribine and pentostatin) were the most frequently used single agents throughout treatment lines. Interferon-alpha represented the first line of treatment in 22% of cases, specifically in those patients treated between 1986 and 1996. Pentostatin was used as a frontline treatment in 6% of the cases, mainly between 1997 and 2001, whereas cladribine was the first choice in 66% of the cases, mostly after 1991. Five percent of patients received splenectomy as their first approach. Significantly, one heavily pre-treated patient received allogeneic bone marrow transplantation as his seventh line.[24]

Summary of treatments and responses

Purine analogues remained the treatment of choice also at first and second disease relapse (cladribine was used in 67% of patients at first relapse and 59% at second relapse, whereas pentostatin represented 7% and 8% of the treatments at first and second relapse, respectively). Rituximab was applied as single agent in 12% of cases during the first salvage setting and in 19% of cases at second disease relapse.

Fifty-four percent of patients required a second treatment (100 out of 184), and again nearly half of those who received a first salvage needed a subsequent line.

Interestingly, the proportion of patients undergoing a new line of treatment was roughly the same throughout the first six treatment lines (range: 54% to 69%, Figure 1). Twenty-five patients overall (13.6%) required more than five lines of therapy, mostly based on new drugs (vemurafenib, moxetumomab pasudotox) or on combinations of rituximab and anti-BRAF agents. Five patients (2.7% of the initial cohort) received an eighth line of therapy. Table 1 is a summary of the agents used.

Overall, 86.4% of patients treated frontline achieved an objective response (CR + PR + minor response), including 43.5% of CR (Figure 1). Similarly, patients receiving a second line had an ORR of 84.0%, with a CR in 38% of the cases. Interestingly, the ORR remained roughly the same within the next four lines of therapy (81.3% for patients receiving a third line, 78.8% for the fourth line, 83.3% for the fifth line and 75.0% for the sixth line), whereas the proportion of patients in CR reduced consistently (37.5% in those receiving a third line, 15.2% for the fourth line, 27.8% and 33.3% for the fifth and sixth line, respectively).

Response obtained with frontline cladribine

Cladribine represented the agent used frontline in 122 (66.3%) patients. In 75 cases (61.5%) it was administered weekly for 5 consecutive weeks; 38 patients (31.1%) received the drug for 5 consecutive days for one single course, while 8 (6.6%) had a course of treatment lasting 7 consecutive days. Administration data were unavailable in one patient. Among cladribine-treated patients, 12 were younger than 40 years (range: 33-39), while six were older than 75 (range: 76-84).

A CR was observed in 66 patients (54.1%), a PR in 32 (26.2%) and a minimal response in 7 (5.7%), yielding an ORR of 86.0%. Among the 66 CR patients, 21 patients received a second treatment line, whereas 45 (68.2% of the CR) were not treated again (Figure 2). More specifically, among the 45 patients in CR who did not received any further

treatment, 28 (42.4% of the CR patients) are maintaining a treatment-free status for 5 years or more. Importantly, in 14 cases the treatment-free interval is longer than 10 years (21.2% of all patients in CR) and in 3 cases it lasts for more than 20 years (4.5% of CR patients).

Among the six elderly patients (age > 75 years), 1 was non-responder, while 5 obtained a PR. Only two of the PR patients underwent a subsequent treatment line, at 3 months and 3 years after the completion of frontline cladribine, respectively. Among the 12 patients younger than 40 years, 8 achieved a CR, 3 a minimal response and 1 was a non-responder. Four out of 8 CR patients are still treatment-free, at a median TTNT of 6.5 years. The remaining four patients in CR required further treatment, at a median TTNT of 4.6 years.

Treatment was overall well tolerated, with a few significant side effects. Interruptions have been documented in 15 cases (12.3% of all treated patients), mainly due to severe neutropenia or febrile neutropenia (5 cases each), sepsis (3 cases), hepatic toxicity or urticarial reaction (1 case each).

Long-term follow up for cladribine-treated patients

The median TTNT for all patients was 8.2 years, with 37.8% of the cases being free of second treatment at 25 years (Figure 3). Median TTNT was not reached for those who achieved a CR after a single course of cladribine, with 52.7% of patients being free of second treatment at 25 years. Conversely, the median TTNT for patients achieving a PR was 5.3 years (P=0.0001). The median OS was not reached, and treated patients have a probability of 65.7% of being alive at 25 years.

Discussion

This is one of the largest single-centre case series gathered in more than thirty years of clinical experience with HCL patients. All the phases of the history of the treatment of this disease are represented, from the initial attempts with splenectomy and interferon-alpha, up to the advent of purine analogues and new agents targeting specific molecules or molecular pathways that characterize the leukemic cells. Cladribine, above all, has emerged as the most frequently used frontline approach in HCL patients requiring therapy, due to its manageability, favorable toxicity profile and efficacy.[25] More than 60% of patients in our series have been treated frontline with cladribine, either once weekly for 5 consecutive weeks or for 5-7 consecutive days, with an ORR of 86.1% and a CR rate of 54.1%. It represents an effective salvage treatment also in those who fail a frontline approach, as it can be repeated even if already used, provided patients display an adequate marrow reserve. We have demonstrated that nearly 50% of patients require further treatment, substantially regardless the number of previous treatment lines they have received: these patients are able to achieve a response even if already treated, although the rate of CR declines progressively as the number of therapies administered previously increases. As in all the chronic lymphoproliferative syndromes, this may be due to a possible biological evolution of the disease, being more chemoresistant as its natural history becomes longer and patients have undergone multiple relapses over time. For this reason, targeted agents – such as the anti-CD22 immunotoxin moxetumomab pasudotox or the orally available BRAF blockers – are good choices in patients who are unlikely to receive purine analogues.[13-15]

The rate of CR we have obtained in our series with cladribine is somewhat different from what has been reported in literature with the same agent, where ORR may reach 100% and CR rates approximate 80-90%.[9,11,26-31] This is mainly due to the fact that we

have decided to refine the concept of CR, conventionally based on the microscopic morphological appearance of a hematoxylin-eosin stained marrow, by applying systematically an immunohistochemistry threshold for CD20 staining to bone marrow specimens. CD20 is brightly expressed on HCL cells and reliably demonstrates any infiltration of the bone marrow by B lymphocytes.[32] A cut-off of 10% of CD20-positive marrow cellularity was established as a positive finding in order to better stratify patients in CR, in view of their long-term prognosis. It is of course worth to note that the use of CD20 as a surrogate for residual marrow disease has some limitations: its expression can be reduced due to its downregulation after any rituximab-containing treatment, for example. This is why combined immunohistochemical stainings, with the association of CD20 with annexin A1 or DBA.44, or PAX5 and CD103, are now being proposed as a way to assess the burden of residual disease.[33,34]

TTNT was chosen as a marker of treatment efficacy over time as it is a descriptor of the proportion of patients who meet the criteria for retreatment at a certain point during their follow-up. It is clear, in fact, that some patients do not require any further treatment, even for a long period of time, even if still displaying active disease, e.g. a certain amount of leukemic infiltration within the bone marrow. TTNT, in our opinion, is much more adequate than progression-free survival (PFS) to describe the behavior of the disease. A correct PFS determination would require serial bone marrow studies – along with peripheral blood assessments – during patients' follow up: this is not a recommended procedure, at least in routine clinical practice, as the amount of disease infiltration within the marrow does not necessarily translate into the development of peripheral blood cytopenias, thus not representing an indicator for retreatment.

It is important to highlight that a substantial proportion of patients treated with frontline cladribine have not required any further treatment: at least 40% of those who achieve a CR are treatment-free for more than 5 years since initial therapy, and roughly a

quarter of them may enjoy treatment-free intervals lasting more than 10 years. This is confirmed by TTNT curves for our series reaching a plateau, particularly as far as patients in CR are concerned. Median TTNT was not reached for CR patients in our series at 25 years, whereas patients achieving a PR are much more likely to require a subsequent line of therapy (as proved by the median TTNT of 5.3 years).

It is hard to understand which patients will do particularly well after one line of cladribine only: perhaps these are patients who have obtained a more profound complete remission rate, which translates into a deeper removal of residual hairy cells both in the peripheral blood and in the bone marrow. In other terms, if on the one hand purine analogues are conceived as the best strategy to control the disease, albeit without its complete eradication, on the other it is possible that a single course of cladribine may be effective in producing an excellent leukemic clearance which can last for many years. In this latter case, patients may be regarded as cured of their disease.[35,36]

Cytofluorimetric or molecular assays performed after frontline treatment, relying on disease-specific antigens (such as CD11c, CD103, CD25, CD123) or disease-driving molecular alterations (as it could be the assessment of the mutated BRAF allele burden) may help to assess the persistence of minimal (or molecular) residual disease, with significantly positive correlations with patients' survival in the very long term.

Conclusions

This single-centre experience displays one of the longest follow-up in literature regarding patients treated with different agents across several treatment eras, as well as with a single purine analogue in a homogeneous way. An attempt to refine the concept of CR by using immunohistochemistry has been proposed and applied, with possible implications on long-term survival rates. We highlight that it is possible to achieve very long-term periods free of disease or treatment, as documented by TTNT curves showing a *plateau* for patients in complete responses after one single cladribine line.

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Author contributions: AB, LA, and PLZ contributed to the design of the study and to work concept. LA performed statistical analyses. CT and ES performed lab tests and collect lab data. AB, VS, LA, MC, BC, AM, GL, GG, CP, LN, PEC, CT, ES and PLZ collected clinical data and contributed to data interpretation. All authors read and approved the final manuscript.

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Table 1. Overview of treatment lines (number of patients).

Drug	**1st line**	**2nd line**	**3rd line**	**4th line**	**5th line**	**6th line**	**7th line**	**8th line**
Cladribine	122	67	28	18	3	2	1	0
Pentostatin	12	7	4	3	3	0	0	1
Interferon	40	11	4	3	2	1	2	0
Rituximab	0	12	9	7	5	4	0	1
Splenectomy	9	3	1	0	1	1	0	0
Vemurafenib	0	0	2	0	1	4	3	0
Moxetumomab pasudotox	0	0	0	1	2	0	1	1
Chlorambucil	1	0	0	0	1	0	0	0
Cobimetinib + vemurafenib	0	0	0	0	0	0	0	1
Steroids	0	0	0	0	0	0	0	1
Rituximab + vemurafenib	0	0	0	1	0	0	0	0
Allogeneic transplant	0	0	0	0	0	0	1	0
	184	***100***	***48***	***33***	***18***	***12***	***8***	***5***

FIGURE LEGENDS

Figure 1. Synthesis of responses according to each treatment line received, regardless the agent used. Arrows among columns indicate the percentage of patients who require a subsequent line of treatment.

Figure 2. Flow chart of patients who had received frontline cladribine. Data are presented according to the depth of the response and its duration.

Figure 3. Time-to-next treatment curves plotted for the entire population of patients receiving frontline cladribine (panel A) and for patients achieving either a complete (CR) or partial response (PR) to frontline cladribine (P=0.0001, panel B). Overall survival curve for all patients receiving frontline cladribine (panel C).