Beyond hairy cell: the activity of cladribine in other hematologic malignancies

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Abstract

Before the contemporary development of rationally designed anti-neoplastic therapies, cladribine was identified as a lymphocyte-specific agent. Its profound impact on the natural history of hairy cell leukemia (HCL), with responses approaching 100% and a median duration of response of nearly a decade following only a single 7-day course, is well known and revolutionized the treatment of HCL. However, cladribine’s impressive activity in other lymphoproliferative disorders has been generally under-appreciated. Multiple single-arm phase II trials have demonstrated cladribine’s potency across the full spectrum of lymphoid malignancies. In a limited number of phase III trials and cross-study analyses, cladribine compared favorably to fludarabine, another purine nucleoside analogue that is more commonly used in the treatment of indolent lymphoid malignancies. Cladribine has been noted to have particular activity among lymphoid disorders with few effective therapies, specifically, chronic lymphocytic leukemia, lymphoplasmacytic lymphoma, marginal zone lymphoma, and mantle cell lymphoma. Recently approved novel agents may act in synergy with cladribine for these conditions and should be incorporated into future clinical studies.
Introduction

Forty years have passed since it was first observed that two girls born with severe combined immunodeficiency disease (SCID) had the unusual findings of both profound lymphocytopenia and undetectable levels of adenosine deaminase (ADA) activity.¹ In 1978, Cohen and colleagues demonstrated that ADA deficiency leads to the toxic accumulation of deoxyadenosine triphosphate (dATP), and subsequent lymphocyte-specific cell death.² Carson postulated that an ADA-resistant purine analogue would be selectively toxic to lymphocytes.³ He identified 2-chlorodeoxyadenosine, 2-CdA, or cladribine (Leustatin, Centocor Ortho Biotech Inc., Horsham, PA) as the most potent ADA-resistant purine nucleoside analogue from a field of candidate congeners.⁴ First synthesized in 1972 by Christensen and colleagues, cladribine closely resembles deoxyadenosine, except for a chlorine atom substitution at the 2-position of the purine ring (Figure 1).⁵

Cladribine’s remarkable activity in hairy cell leukemia (HCL), producing an overall response rate (ORR) of 98% with 91% complete responses (CR), and extremely prolonged remissions following only a single 7-day course with a very favorable toxicity profile, is widely known.⁶ Less well promulgated is cladribine’s role in the treatment of non-HCL indolent lymphoproliferative disorders. This review will focus on the considerable body of literature evaluating cladribine in these indolent lymphoid malignancies and propose a strategy for its further development in combination with novel agents.

Mechanism of action

Like deoxyadenosine, cladribine enters lymphocytes through an efficient transport mechanism (Figure 2).⁷,⁹ Once in the cell, deoxyadenosine has two potential fates.
Deoxyadenosine is subject to either irreversible deamination by ADA, leading ultimately to the uric acid excretion pathway, or serial phosphorylation by deoxycytidine kinase (DCK) to dATP. Cladribine, on the other hand, is resistant to deamination by ADA because of its chlorinated purine ring structure and instead becomes phosphorylated by DCK to its lymphocytotoxic form, 2-chlorodeoxyadenosine triphosphate. The intracellular concentrations of these phosphorylated purines are regulated by the competing kinetics of DCK, a phosphorylating enzyme, and 5’-nucleotidase (5’-NT), a dephosphorylating enzyme. Lymphocytes are unique for constitutively expressing high levels of DCK, such that the DCK to 5’-NT ratio favors phosphorylation, making them particularly susceptible to the actions of cladribine. Cladribine resistance has been correlated with DCK levels downregulated to 5% or less, and 5’-NT levels 200% of normal, altering the DCK:5’NT ratio, which then favors drug dephosphorylation. Decreased nucleoside transport, altered ribonucleotide reductase (RNR) regulation, and defective apoptotic pathways have also been implicated in cladribine resistance. Although non-HCL lymphocytes can have an elevated DCK:5’-NT ratio and are very sensitive to cladribine, it is unclear why cladribine’s effect is most durable in HCL.

Cladribine is cytotoxic to both dividing and non-dividing lymphocytes from both healthy donors and patients with lymphoproliferative disorders. In resting cells, cladribine causes single-strand DNA breaks, inducing the DNA repair enzyme, poly(ADP-ribose) polymerase or PARP. PARP expression exhausts the intracellular pools of nicotinamide adenine dinucleotide (NAD) and adenosine triphosphate (ATP), precipitating apoptotic cell death. Recently, cladribine has also been shown to induce apoptosis by altering the mitochondrial transmembrane potential, enabling the translocation of cytochrome c and apoptosis-inducing factor into the cytosol, causing apoptosis in both a caspase-dependent and independent process. In dividing
cells, cladribine induces cytotoxicity by impairing DNA synthesis via two key mechanisms: first, it potently inhibits RNR, and second, it competes with dATP for incorporation into DNA by DNA polymerases α and β. Preclinical and clinical data suggest that cladribine may also have hypomethylating activity via its indirect inhibitory effects on DNA methyltransferase and depletion of the methyl donor pool.

**Pharmacokinetics**

With plasma protein binding of only 20%, cladribine has a wide volume of distribution, specifically, 9 L/kg. Cladribine is excreted unchanged through the kidneys. Its elimination kinetics best fits a two-compartment model with α and β half-lives of 35 minutes and 6.7 hours, respectively. Administering 0.14 mg/kg of cladribine as a 24 hour continuous intravenous (IV) infusion produces a mean steady state concentration of 22.5 nM. Given as a 2-hour bolus at 0.14 mg/kg, the mean peak plasma concentration reaches 198 nM, and falls to 22.5 nM within six hours after completing the bolus. Two-hour bolus and infusional cladribine have comparable areas under the curve (AUC) of 588 and 552 nM x h, respectively. The intracellular concentration of cladribine is known to be higher than the plasma concentration.

Alternative routes of administration have been explored. Subcutaneous (SQ) dosing (0.14 mg/kg) demonstrates equal bioavailability, volumes of distribution, and excretion profiles compared to an equivalent dose administered as a 2-hour IV bolus. Interestingly, rapid vascular uptake after SQ dosing yielded a peak serum concentration greater than the 2-hour IV bolus, whose duration of infusion exceeded cladribine’s brief biological half-life, blunting its peak serum concentration. An oral dose of 0.28 mg/kg, double the typical IV dose, achieves similar AUC values as IV and SQ dosing and has a bioavailability of 55%. Cerebral spinal fluid (CSF)
concentrations appear non-linear relative to serum drug concentrations with markedly increased levels when cladribine is dosed above 0.1 mg/kg/day. At the 0.15 mg/kg/day continuous infusion regimen, the mean CSF concentration of cladribine reaches 20 nM/L, twice the in vitro ID50 for lymphoblast cell lines.25

Preclinical and phase I results

Cladribine selectively inhibits the growth of malignant human T-, B-, and null lymphoblastoid cell lines at nanomolar concentrations and is also lethal to resting lymphocytes. T-cell lines are as much as one log more sensitive to cladribine than B-cell lines.10 At equivalent concentrations, cladribine has almost no effect on solid tumor cell lines. The L1210 murine leukemia model demonstrates improved survival in cladribine treated mice compared to mice receiving placebo or even fludarabine, a related purine nucleoside analog.4

The first phase I trial of cladribine began in 1981 at Scripps Clinic under the direction of Dr. Ernest Beutler. Nine patients with refractory advanced hematologic malignancies received cladribine by continuous IV infusion at doses of 0.1-1 mg/kg/day for 5-14 days with dose escalations of 0.1 mg/kg/day for those without an initial therapeutic or toxic response. Cladribine lowered the blast count in all of the leukemic patients by a minimum of 50%. Dose-limiting toxicities included marked leukopenia and thrombocytopenia at doses above 0.15 mg/kg/day. This study established the maximum tolerated dose of infusional cladribine to be 0.1 mg/kg/day for 7 days.15

Indolent non-Hodgkin lymphoma (NHL)
Indolent NHL constitutes a wide spectrum of B-cell histologies. Follicular lymphoma is the most common subtype in the United States, and is frequently treated with rituximab in combination with cyclophosphamide, vincristine, and prednisone. Marginal zone and lymphoplasmacytic lymphomas, while less common, generally have excellent initial responses to fludarabine. Mantle cell lymphoma (MCL) has fewer, and generally unsatisfactory, available treatments. Standard curative therapies do not exist for any of the indolent lymphomas, and additional treatment options are needed. A large number of clinical studies indicate that cladribine has activity across the full spectrum of indolent lymphoma subtypes and can be a useful addition to the therapeutic armamentarium.

**Single agent therapy in previously treated indolent NHL**

Eleven phase II studies have evaluated single agent cladribine in the setting of relapsed or refractory indolent lymphomas (Table 1).26-36 Cladribine demonstrated impressive activity with overall and complete response rates of up to 72% and 38%, respectively. Follicular and non-follicular histologies appeared to be equally responsive. In the first of these studies, 40 relapsed and refractory low-grade lymphoma patients with a median of 3 prior therapies were treated with cladribine at 0.1 mg/kg/day by continuous infusion for 7 consecutive days. Two-thirds of these patients had follicular histology. The overall response rate (ORR) was 43% (17 patients) with a complete response rate of 20% (8 patients) and a 5-month median duration of response.26 A 2-hour bolus regimen produced similar results in a comparable patient population.27 These results were later reproduced in a trial where 80% of patients had a non-follicular histology, mainly lymphoplasmacytic lymphoma (37%) or B-CLL/small lymphocytic lymphoma (29%).28 Of 94 patients, 48 (51%) achieved a response and 12 (13%) had a CR. The median duration of
response was 12 months for a CR and 6 months for a PR. These non-randomized trials clearly documented that cladribine can be highly active among pretreated patients and should be considered in this clinical setting. However, these patients were not exposed to the current therapeutic landscape, including rituximab, autologous stem cell transplant, and emerging novel agents, such as bendamustine and bortezomib. Only future randomized studies incorporating cladribine will be able to determine an optimal approach.

**Combination therapy in previously treated indolent NHL**

Attenuated doses of cladribine have been safely combined with other chemotherapeutic agents in patients with previously treated indolent lymphomas achieving promising results. Studies evaluating cladribine in combination with cyclophosphamide, mitoxantrone, or rituximab reported superior response rates compared to single agent cladribine (Table 2).\(^{37-45}\) However, as is often the case in studies with indolent lymphomas, there were no statistical differences in overall survival. Toxicities were typically hematologic and infectious, and were generally not severe. A single study cautioned against the routine use of infusional cladribine with cyclophosphamide as dose-limiting toxicities occurred in over half the patients, with a third of patients experiencing autoimmune phenomena, including autoimmune hemolytic anemia, immune thrombocytopenic purpura, and pure red cell aplasia. However, one of the cohorts in the study represented a dose-finding arm, possibly explaining some of the observed toxicities.\(^{37}\)

**Single agent therapy in previously untreated indolent NHL**

After demonstrating major activity in patients with heavily pre-treated indolent lymphomas, single agent cladribine was subsequently introduced in the front-line setting. As
was the case for single-agent cladribine in previously treated patients, most patients had a follicular histology, with follicular and non-follicular histologies generally demonstrating similar outcomes. Patients had OR and CR rates of up to 98% and 38%, respectively, comparing favorably to single agent cladribine in the salvage setting. The median duration of response varied widely from 7 to 23 months (Table 3).46-52

The CALGB conducted the most recent single arm phase II trial, enrolling 44 treatment naive indolent lymphoma patients, the majority (73%) of whom had a follicular subtype. Patients received bolus cladribine at 0.14 mg/kg/day over 2 hours for 5 consecutive days every 28 days for up to 6 cycles, achieving an ORR of 98% (43 patients) and 32% CRs (14 patients). The median response duration was 1.3 years for PRs and 3.1 years for CRs, with a median overall survival of 7 years.46 Table 3 lists the other 6 reported cladribine studies in untreated indolent lymphoma patients, demonstrating its consistent activity across trials. Toxicity in the CALGB study was considerable with 68% experiencing a grade 3 or 4 event, largely myelosuppression, and 8 patients had grade 3 infections. Four histologic transformations to high-grade lymphoma were documented, between 2 and 32 months following the last cycle of cladribine.46

Non-follicular histologies appear equally sensitive to cladribine (Table 4).45,53-60 The largest single arm phase II cladribine study in this setting enrolled 66 patients (80% untreated) with a variety of histologies, including follicular (40%), lymphoplasmacytic (30%), and MCL (20%). Even though half of the MCL patients were pre-treated, that group achieved a 58% response rate with 25% CRs.47,53 Similarly, a study of 26 treatment-naive gastric marginal zone lymphoma patients reported an ORR of 100% with 84% CRs. Of 7 patients with an extra-gastric presentation, 3 patients achieved a CR. Of note, a fatal myocardial infarction and stroke were reported within days and 3 months of the first and third course of therapy, respectively.54 A
small series of splenic marginal zone patients in relapse after chlorambucil demonstrated an ORR of 86%, but with a short duration of remission and significant infectious complications.\textsuperscript{55}

Despite cladribine’s demonstrated activity in untreated indolent NHL, there has only been one published phase III study to date. The Polish Lymphoma Research Group randomly assigned 197 untreated indolent lymphoma patients to one of three monthly treatment arms: single-agent cladribine, cladribine plus cyclophosphamide, or cyclophosphamide, vincristine and prednisone (CVP) (Table 5).\textsuperscript{61} Histologic subtypes by WHO classification included chronic lymphocytic leukemia (CLL), 35%; follicular, 28%; marginal zone, 25%; and lymphoplasmacytic, 7%. The primary endpoint was progression-free survival (PFS). At 10 months, accrual to the CVP arm was prematurely discontinued due to statistically superior PFS (p<0.0001) and ORR (p<0.0001) in the cladribine containing arms. At 3-year follow-up, PFS for combination cladribine and cyclophosphamide, single-agent cladribine, and CVP was 61%, 48%, and 22%, respectively. Statistical differences were observed between the CVP and the cladribine containing arms, but not between the cladribine containing arms. However, the combination cladribine and cyclophosphamide arm did report a statistically greater number of CRs than single-agent cladribine. No statistical differences were found in overall survival. In a multivariate analysis with International Prognostic Indices, cladribine therapy remained an independent prognostic factor for improved PFS. Endpoints for all histologic subgroups were reported as being similar. Infections occurred in 7% of patients in each arm, but grade 3 hematologic toxicity was most common in the combination cladribine and cyclophosphamide arm.\textsuperscript{61}

Cladribine has consistently demonstrated impressive activity across a wide spectrum of indolent lymphomas in untreated patients. Except for its myelosuppressive qualities, it is well
tolerated, and can be considered as front-line single-agent therapy in appropriate indolent lymphoma patients with a good performance status. In fact, cladribine was superior to CVP chemotherapy, another standard treatment, for response and survival endpoints in a study that mirrored a similar trial comparing single-agent fludarabine to CVP. However, with the superiority of rituximab-CVP compared to CVP alone and the promising results of rituximab-bendamustine, optimal front-line treatment of indolent lymphoma is in constant flux.

**Waldenström macroglobulinemia**

Waldenström macroglobulinemia (WM) is a malignant B-cell lymphoplasmacytic lymphoma characterized by pathologic monoclonal IgM immunoglobulin overproduction. Several studies have evaluated cladribine in previously treated and untreated patients. In one study, 26 untreated symptomatic patients were given cladribine 0.1 mg/kg/day by 7-day continuous infusion and obtained an ORR of 85% with a median response duration of over 13 months. Other reports corroborated the sensitivity of WM to cladribine, with response rates ranging from 41 to 90% and a median duration of response between 9 to 18 months (Table 4). These results are comparable to front-line fludarabine studies in similar patient populations, producing response rates from 38 to 100%. In its most recent update, the International Workshop on Waldenström Macroglobulinemia recommended cladribine as an acceptable first-line treatment for WM, either as a single-agent or in combination with rituximab or cyclophosphamide. However, its use should probably be avoided in younger patients as recent reports have noted a potentially increased risk of Richter’s transformation, myelodysplasia, and acute myeloid leukemia in WM patients receiving a purine nucleoside analogue.
Mantle cell lymphoma

MCL can exhibit an indolent course, but most patients eventually require therapy for symptomatic progression. Even patients receiving the most aggressive combination chemotherapy or transplant programs will relapse within a few years. Several single-arm studies with mixed populations of previously treated and untreated patients suggest that cladribine has considerable activity in MCL (Table 4). Twelve patients with MCL (7 previously treated) were administered cladribine 5 mg/m²/day as a 2-hour bolus for 5 consecutive days, yielding an ORR of 58% (7 patients) with CRs in 25% (3 patients), and a median time to progression of 19 months. The North Central Cancer Treatment Group administered the same cladribine schedule to patients with MCL, and documented an ORR of 81% (21 patients) with 42% CRs (11 patients) among 26 treatment naïve patients and an ORR of 46% (11 patients) with 21% CRs (5 patients) for 24 previously treated patients.

Combination approaches in previously treated and untreated MCL patients have also documented significant clinical activity. Among 29 previously untreated MCL patients administered combination cladribine and rituximab, there was an ORR of 66% (19 patients) with 52% CRs (15 patients). After 21.5 months of follow-up, 80% of patients in the combined treatment arm who had achieved a CR were still in remission. In another trial, of 18 MCL patients treated with combination cladribine and mitoxantrone, all responded (8 patients with a CR), and had a 24 month median duration of response. Among a different group of nine MCL patients given cladribine in combination with cyclophosphamide and rituximab, 6 patients responded with 2 obtaining a CR.

These results demonstrate that cladribine is highly active both as a single agent and as a component of combination therapy for MCL patients. Single-agent use of cladribine may be
appropriate in selected patient populations, but the incorporation of cladribine into future multi-agent approaches will probably be most effective.

**Chronic lymphocytic leukemia/small lymphocytic lymphoma**

Chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma can be considered overlapping pathologic and clinical entities that are distinguished from each other by the presence of lymphadenopathy and lymphocytosis.\(^66\) Fludarabine has become the cornerstone of first-line CLL therapy, either as a single-agent or in combination with cyclophosphamide and/or rituximab.\(^67,68\) Cladribine’s role in treating CLL has been largely overlooked, despite being a related purine nucleoside analogue to fludarabine, with impressive clinical results. In preclinical studies, CLL B-lymphocytes undergo apoptosis when incubated with cladribine via mitochondrial membrane disruption, releasing proapoptotic cytochrome c and apoptosis-inducing factor.\(^69,70\) Multiple single-arm and randomized clinical trials have confirmed cladribine’s major activity in CLL (Table 6).\(^71-80\)

**Single agent therapy in previously treated CLL**

Piro and colleagues first evaluated cladribine in previously treated CLL patients, documenting an ORR of 50% in 18 patients.\(^81\) Continued enrollment to this trial ultimately accrued 90 refractory CLL patients, administering cladribine as either a 0.1 mg/kg/day 7-day continuous infusion or as a 0.028-0.14 mg/kg/day 2-hour bolus for 5 consecutive days, producing an ORR of 44% (40 patients) with 4% CRs (4 patients). Of those patients not meeting response criteria, over half achieved a 50% or greater sustained reduction in the absolute lymphocyte count.\(^71\) A trial of bolus cladribine dosing in 92 previously treated patients reported remarkably
similar results (Table 6). Patients in these two studies each had a median of 2 prior therapies, suggesting that single-agent cladribine was an effective salvage therapy in CLL. However, because these patients only received alkylating agents or steroids, and not modern combination therapies that incorporated fludarabine and rituximab, the role of single-agent cladribine in the current therapeutic arsenal of CLL salvage therapy is less clear. Small studies that have evaluated the activity of cladribine in fludarabine-refractory CLL patients, or the reverse, suggest that cross-resistance exists (see “Cladribine versus fludarabine”), and that salvage cladribine in purine analogue-refractory patients may actually be deleterious. In contrast, among patients who relapsed, but were not refractory, after prior purine analogue therapy (either cladribine or fludarabine), cladribine has activity and may be considered in this scenario.34,82,83

**Combination therapy in previously treated CLL**

In the salvage setting, combination cladribine therapies have not been clearly shown to offer clinically significant advantages over single-agent cladribine use (Table 6). A group of heavily pretreated CLL patients (median prior chemotherapy courses, 7.5; range, 5-14) were given cladribine at a dose of 0.12 mg/kg/day by 2-hour bolus for 5 days in combination with cyclophosphamide and mitoxantrone. Responses were not superior to the historical reports for single agent cladribine (37% ORR, 19 patients) but toxicities were considerable, including severe neutropenia and infections/fever of unknown origin (FUO) in up to 50% and 83% of patients, respectively.73 A randomized study of cladribine in combination with prednisone versus cladribine alone also did not find a significant difference in response rates, although the combination arm had improved median OS (14 months versus 10 months, p=0.001). The authors hypothesized that the survival advantage had resulted from a salutary effect of steroids on bone
marrow function and CLL-induced autoimmunity, but ultimately concluded that combination cladribine and prednisone therapy was not warranted except for pre-treated patients with severe anemia or thrombocytopenia.\textsuperscript{74}

Cladribine has been administered together with rituximab in 15 relapsed/refractory CLL patients producing an impressive ORR of 73\% (11 patients) with 7\% CRs (1 patient), providing the rationale for additional cladribine-rituximab combination approaches.\textsuperscript{75} However, with the current standard practice of incorporating rituximab into up-front CLL therapy, subsequent rituximab-cladribine combinations may prove less valuable.

**Single agent therapy in previously untreated CLL**

Early studies evaluating front-line cladribine in untreated CLL patients produced an ORR ranging from 72-85\% with a CR range of 25-37\%.\textsuperscript{74,84,85} These results seem to be similar, or even more impressive, than outcomes from studies using single-agent fludarabine, and are comparable to results reported for combination fludarabine and cyclophosphamide regimens.\textsuperscript{67} In the only prospective phase III trial evaluating cladribine in the front-line setting, 229 patients were randomized to cladribine, fludarabine, or high-dose intermittent chlorambucil. Cladribine was administered at 5 mg/m\textsuperscript{2}/day as a 2-hour bolus for 5 consecutive days. No statistical differences in ORR or OS were detected among the treatment arms, however, cladribine produced a significantly improved median time to progression of 25 months versus 10 months for fludarabine, and 9 months for chlorambucil (p=0.0003)\textsuperscript{86} (Table 7).\textsuperscript{86-89} For patients who are not candidates to receive combination chemotherapy regimens, administering single-agent cladribine is therefore a reasonable option.
**Combination therapy in previously untreated CLL**

In a randomized trial, combination cladribine and prednisone produced improved response rates and PFS, but no difference in OS, when compared to combination chlorambucil and prednisone or single-agent cladribine. A three drug combination of cladribine, mitoxantrone, and cyclophosphamide was compared to single-agent cladribine, with or without cyclophosphamide, producing increased CRs, but no differences in ORR, PFS, or OS. The three drug combination reported increased myelotoxicity and 40% of patients developed severe infections (Table 7). The nominal improvements in clinical endpoints derived from the cladribine, cyclophosphamide, and mitoxantrone combination treatment probably do not justify further investigational consideration of this regimen. Administering combination cladribine in sequence, instead of concurrently, offered a highly effective and potentially less toxic approach.

**Cladribine versus fludarabine**

Cladribine’s ability to induce apoptosis by altering the mitochondrial membrane potential is not shared by fludarabine and may serve as a non-overlapping mechanism of action, potentially allowing cladribine’s use to be exploited in certain clinical scenarios. Four fludarabine-refractory CLL patients were reported to have responded to subsequent cladribine therapy. However, far more clinical data exists to support cross-resistance between these 2 purine nucleoside analogues. Only 2 of 10 cladribine-refractory patients responded to second-line fludarabine. In another report, only 7% (2 patients) of fludarabine-refractory patients had a response to cladribine. In the sole randomized study, 60 patients with relapsed or refractory indolent lymphoma were assigned to either single-agent cladribine or fludarabine and then later
crossed-over to the alternative purine analogue at progression. At cross-over, 8 of 9 patients with an initial response responded again, but refractory patients did not, also suggesting cross-resistance.\textsuperscript{34}

Despite the current trend favoring fludarabine-based therapies for CLL, it is not clear that front-line fludarabine is necessarily superior to cladribine. A randomized phase III study comparing single-agent fludarabine to cladribine in front-line CLL therapy noted a markedly superior median time-to-progression of 25 months for cladribine versus 10 months for fludarabine (p=0.0003).\textsuperscript{86} Final analysis of the PALG-CLL3 study that administered cyclophosphamide in combination with either cladribine or fludarabine to previously untreated CLL patients reported equivalent CR, ORR, and PFS with comparable toxicities.\textsuperscript{89} These two randomized reports indicate that cladribine is at least equivalent to fludarabine in the front-line therapy of CLL. Cross-study comparisons of either the fludarabine-cyclophosphamide arm or cladribine-cyclophosphamide arm from other front-line phase III CLL trials reinforce this conclusion (Table 8).\textsuperscript{67,88,89,93,94}

Fludarabine-based therapies in CLL have been unable to overcome the deleterious influence of the 17p13.1 deletion.\textsuperscript{93} Cladribine’s ability to produce caspase-dependent apoptosis by altering the mitochondrial membrane potential, a p53-independent pathway, could potentially permit it to overcome the negative effects of the 17p13.1 deletion.\textsuperscript{89} A retrospective review of 20 patients with 17p13.1 deletions treated with front-line cladribine-cyclophosphamide noted an ORR of 80% (16 patients) with 50% CRs (10 patients) and a median PFS of 23 months.\textsuperscript{95} Final results of the PALG-CLL3 study reported a non-significant improvement in the CR rate for the cladribine arm of the 17p-deleted subgroup (40% versus 15%, p=0.112). However, a random nonbalanced distribution of the 17p-deleted patients placed more advanced stage patients in the
fludarabine-based cohort, undermining this promising trend.\textsuperscript{89} Opportunity for improved therapies in this poor-prognosis setting remains an open area for future trials.

**Acute Myeloid Leukemia**

Although the focus of this review is on the indolent lymphoproliferative disorders, it is important to mention recent cladribine data documenting its activity in acute myeloid leukemia (AML), a disease in urgent need of new therapeutic options. Seventeen children with refractory AML were treated with cladribine at $8.9 \text{mg/m}^2$/day for 5 days via continuous infusion, and 8 obtained a CR.\textsuperscript{96} When AML patients older than 40 years were randomized between induction daunorubicin, cytarabine, and cladribine (DAC) versus daunorubicin and cytarabine (DA) alone, patients treated with DAC had a 17\% higher CR rate and statistically improved OS (26\% DAC vs 15\% with DA alone, $p=0.03$).\textsuperscript{97} An abstract from the American Society of Hematology 2009 annual conference compared DAC to DA plus fludarabine (DAF) and standard DA in 673 adult untreated AML patients. The CR rate was significantly superior in the DAC arm (68\% DAC vs 59\% DAF and 56\% DA, $p=0.013$ and $p=0.08$, respectively) as was the three year OS (46\% DAC vs 30\% DAF and 31\% DA, $p=0.02$).\textsuperscript{98} These encouraging results provide a foundation to design future studies utilizing cladribine as a component in the induction phase of AML therapy.

**Miscellaneous conditions**

Cladribine has also been reported to have activity in a number of much less common hematologic neoplasms, including Langerhans cell histiocytosis\textsuperscript{99}; systemic mastocytosis\textsuperscript{100}; hypereosinophilic syndromes\textsuperscript{101}; myelofibrosis and myeloid metaplasia\textsuperscript{102}; cutaneous T-cell lymphoma\textsuperscript{103}; and B-cell prolymphocytic leukemia\textsuperscript{104,105}.
Cladribine Toxicities

Cytopenias

Myelosuppression is cladribine’s principal toxicity (Table 9). Patients with CLL and indolent lymphoma experience similar rates and grades of neutropenia, thrombocytopenia, and anemia. The two most commonly administered cladribine regimens, 2-hour bolus and 7-day continuous infusion, also have comparable toxicities. In the front-line setting, grade 3/4 neutropenia and thrombocytopenia occurred in up to 60% of patients, with anemia only reported in up to 13%. Infection or FUO developed in up to 45% of patients. Cross-study comparisons demonstrate that pre-treated patients given cladribine had similar rates of myelotoxicity and infections as previously untreated patients. Repetitive administration of cladribine can result in cumulative myelosuppression, which limited many patient cohorts to only three or four cycles of the drug. Instead of analyzing bone marrow cellularity prior to the initiation of subsequent cycles, it is our practice to simply delay therapy until a patient’s absolute neutrophil count has recovered to ≥ 1,000/mm³ and the platelet count to ≥ 100 × 10⁹/L.

Cytopenias can persist for greater than 2 months, and have very rarely been associated with thrombocytopenia-induced retinal hemorrhage, pericardial tamponade, and the death of at least one patient from intracerebral hemorrhage. Fatal neutropenic sepsis has been reported in pre-treated patients receiving cladribine.
Opportunistic infections

Bacterial and opportunistic infections (OI) were the most frequent non-hematologic toxicities (Table 9). Marked and prolonged T-cell lymphocytopenia, in addition to monocytopenia and neutropenia, may explain this increased infectious risk. Cladribine has been found to suppress both CD4 and CD8 levels and reduce the CD4/CD8 ratio, lasting from 12 to 54 months.\textsuperscript{107} Identified OIs include cytomegalovirus, herpes simplex, dermatomal and disseminated herpes, \textit{Pneumocystis carinii}, listeriosis, and disseminated fungi. Delayed infections, some occurring up to 31 months after cladribine treatment, occurred in previously treated and untreated patients and resulted in some patient deaths. \textit{Mycobacterium tuberculosis} reactivation was reported in 2 patients.\textsuperscript{106} Deaths have also been attributed to interstitial pneumonia and generalized herpes simplex virus infection.\textsuperscript{35}

By extrapolating from the recommendations for antiviral and \textit{Pneumocystis} prophylaxis in patients treated with fludarabine, cyclophosphamide, and rituximab combinations, patients administered cladribine-based combination therapy should receive similar prophylactic antimicrobials, including standard prophylactic doses of bactrim and acyclovir.\textsuperscript{108} Caution should be exercised in utilizing cladribine and prednisone combinations due to an increased risk for opportunistic and serious infections when purine analogues and steroids are used together.\textsuperscript{109} Due to the delayed nature of some of these infections, prophylaxis should continue for an extended period of time after treatment discontinuation.\textsuperscript{108}
Secondary malignancy risk

Cladribine’s immunosuppressive and DNA damaging properties introduce the possibility of delayed treatment-induced secondary malignancies, especially when combined with other alkylating agents, such as cyclophosphamide. Several analyses have documented myelodysplasia in cladribine-treated patients, with one series reporting an incidence of 1.6%.45,110,111 Anecdotal experience at Scripps Clinic suggests that the risk of myelodysplasia may be even higher and that cladribine use can be a myelodysplasia risk factor. The exact mechanism and association remain speculative, although cladribine is incorporated into replicating DNA.110,111

A review of 1,487 patients treated with either cladribine, an alkylating agent, or their combination did report a statistical increase in lung cancer incidence among cladribine-based therapy, but the authors made no definitive conclusions.112 A comparison of purine analogue treated patients to an age-adjusted population from the SEER database found a slight increase in observed-to-expected secondary malignancies. However, this finding was more consistent with the known increased incidence associated with lymphoproliferative disorders and not necessarily from purine analogue exposure.113

Autoimmunity

Although autoimmune hemolytic anemia (AIHA) has been associated with cladribine, randomized studies have been unable to absolutely associate cladribine use with the development of AIHA.114-117 CLL patients randomly assigned to cladribine did not have a significant increase in AIHA compared to patients treated with chlorambucil.87 It may be that the underlying lymphoproliferative disorder itself represents the major risk factor for AIHA rather than the
cladribine treatment. Nevertheless, the generally advocated approach is to avoid purine nucleoside analogues in patients with established AIHA or ITP.

Pure red cell aplasia (PRCA) also only has a tenuous association with cladribine. A large retrospective review of 470 CLL patients identified only 8 patients with PRCA, 5 following cladribine therapy and 3 diagnosed prior to therapy. The incidence of PRCA in patients treated with single-agent cladribine was not greater than other CLL treatments described in the literature.\textsuperscript{118}

**Rare toxicities**

Several other rare, but documented, toxicities include Stevens-Johnson syndrome/toxic epidermal necrolysis, stroke, tumor lysis syndrome, and transfusion-associated graft versus host disease.\textsuperscript{76,106} At approved doses, cladribine has been reported to produce a mild to moderate, but generally reversible, neuropathy in 10 to 15\% of patients.\textsuperscript{119} This risk is not thought to be high since cladribine has been successfully applied to the treatment of multiple sclerosis.\textsuperscript{120} One patient with a preexisting paraneoplastic syndrome died of a rapidly progressive sensorimotor peripheral neuropathy after completing 2 courses of low-dose cladribine (0.1 mg/kg/day 2-hour infusion for 7 days).\textsuperscript{121} In addition, at very elevated doses (more than triple the approved dose) a delayed severe motor weakness developed.\textsuperscript{122}

**Future Research Proposals**

Cladribine’s most influential role in the treatment of indolent lymphoid malignancies will likely be as a component of front-line multi-agent therapy. Previous attempts to combine cladribine with standard cytotoxic agents, such as cyclophosphamide and mitoxantrone, resulted
in significant toxicities with only minor advantages over single agent cladribine. Instead, regimens that incorporate cladribine with recently developed novel agents appear to be mechanistically the most promising research direction to take. These newer agents include lenalidomide and bortezomib, both offering broad activity across the spectrum of indolent lymphoma subtypes, non-overlapping mechanisms of action compared to cladribine, and can be used in combination with rituximab. Another interesting hypothesis is to combine cladribine with one of the newly developed PARP inhibitors. Cladribine can trigger apoptosis by stimulating PARP expression which depletes intracellular NAD and ATP (see “Mechanism of action”), and PARP inhibitors may contribute to this process. However, a comprehensive pre-clinical analysis of this approach must be performed prior to its clinical implementation as there is also a potential for cell rescue if the PARP inhibitor prevents nucleotide depletion.

A further clinical trial proposal is the addition of bendamustine to cladribine together with rituximab. Bendamustine is a molecule that has a purine-like ring, but acts as an atypical alkylating agent. As a purine nucleoside analogue, cladribine may provide valuable structural synergies to bendamustine. Finally, rituximab has been safely and successfully used with both cladribine and bendamustine. Although this approach may cause significant myelosuppression, cladribine and bendamustine are among the most effective single-agent therapies for both CLL and MCL, possibly making this a highly effective regimen.

Conclusions

Despite its spectacular success in treating HCL, cladribine has not been a widely adopted therapy for other lymphoproliferative disorders. We have summarized the literature demonstrating cladribine’s remarkable activity in indolent lymphoid malignancies other than
HCL, as well as in AML more recently. Cladribine induces clinically significant responses across a range of indolent lymphomas and CLL, among both untreated and previously treated patients. Lymphoplasmacytic lymphoma (Waldenström macroglobulinemia) and marginal zone lymphoma appear particularly sensitive to cladribine’s action. Patients with MCL, an aggressive lymphoproliferative disorders with limited therapeutic options, experienced durable responses after cladribine therapy. Cross-study comparisons and a phase III study in CLL suggested that cladribine may confer therapeutic advantages over fludarabine, supporting the continued clinical development of cladribine. Standard cladribine dosing is generally well-tolerated, mainly causing cytopenias and fevers.

This review highlights cladribine’s role in the management of patients with indolent lymphomas, however, the majority of these studies were small in size and included heterogenous patient populations. In addition, the most appropriate dosing regimens and combination approaches have not been clearly defined. A mechanistic review suggests that cladribine may have synergy with some recently approved novel agents. Clearly, cladribine’s promising role in indolent lymphomas, CLL, and AML has not been fully explored. It is incumbent on investigators in hematologic malignancies to be familiar with cladribine’s unique activity and advantages in these disease states so that its future inclusion in clinical research protocols can be best exploited to advance patient care.
Author Contributions

DSS and AS conceived of the paper. DSS composed the manuscript and compiled literature data. HM and ES compiled medical literature data, prepared tables and figures, and contributed to manuscript composition. AS edited the manuscript.

The authors have no conflict of interest.

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Table 1. Phase II studies of single agent cladribine in previously treated indolent non-Hodgkin lymphoma

<table>
<thead>
<tr>
<th>Study</th>
<th>Cladribine Doses</th>
<th>Histology (%)</th>
<th>Prior Therapies</th>
<th>Median Response (%)</th>
<th>Median Duration (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kay et al 1992\textsuperscript{26}</td>
<td>A 0.1 mg/kg/day</td>
<td>Follicular 65</td>
<td>Other 35</td>
<td>3</td>
<td>43 20</td>
</tr>
<tr>
<td>Hickish et al 1993\textsuperscript{29}</td>
<td>A 0.14 mg/kg/day</td>
<td>Follicular 82</td>
<td>Other 18</td>
<td>4</td>
<td>71 29</td>
</tr>
<tr>
<td>Hoffman et al 1994\textsuperscript{30}</td>
<td>A 0.12 mg/kg/day</td>
<td>Follicular 25</td>
<td>Other 75</td>
<td>2</td>
<td>45 15</td>
</tr>
<tr>
<td>Morton et al 1996\textsuperscript{27}</td>
<td>B 0.09 mg/kg/day</td>
<td>Follicular 67</td>
<td>Other 33</td>
<td>2</td>
<td>63 11</td>
</tr>
<tr>
<td>Liliemark et al 1997\textsuperscript{31}</td>
<td>C 0.12 mg/kg/day</td>
<td>Follicular 47</td>
<td>Other 53</td>
<td>2</td>
<td>42 14</td>
</tr>
<tr>
<td>Robak et al 1997\textsuperscript{28}</td>
<td>C 0.12 mg/kg/day</td>
<td>Follicular 18</td>
<td>Other 82</td>
<td>4</td>
<td>51 13</td>
</tr>
<tr>
<td>Kong et al 1998\textsuperscript{32}</td>
<td>A 0.12 mg/kg/day</td>
<td>Follicular 55</td>
<td>Other 45</td>
<td>3</td>
<td>45 9</td>
</tr>
<tr>
<td>Tulpule et al 1998\textsuperscript{33}</td>
<td>B 0.14 mg/kg/day</td>
<td>Follicular 54</td>
<td>Other 46</td>
<td>2</td>
<td>31 12</td>
</tr>
<tr>
<td>Tondini et al 2000\textsuperscript{34}</td>
<td>B 0.09 mg/kg/day</td>
<td>Follicular 57</td>
<td>Other 43</td>
<td>2</td>
<td>72 38</td>
</tr>
<tr>
<td>Ogura et al 2004\textsuperscript{35}</td>
<td>D 0.1 mg/kg/day</td>
<td>Follicular 80</td>
<td>Other 20</td>
<td>2</td>
<td>58 14</td>
</tr>
<tr>
<td>Tobinai et al 2009\textsuperscript{36}</td>
<td>C 0.1 mg/kg/day</td>
<td>Follicular 72</td>
<td>Other 28</td>
<td>2</td>
<td>50 11</td>
</tr>
</tbody>
</table>

CR: complete response; OR: overall response
A: 0.1 mg/kg/day as continuous IV infusion for 7 days
B: 0.14 mg/kg/day as 2-h IV infusion for 5 consecutive days
C: 0.12 mg/kg/day as 2-h IV infusion for 5 consecutive days
D: 0.09 mg/kg/day as continuous IV infusion for 7 days
### Table 2. Phase II studies of cladribine combinations in indolent non-Hodgkin lymphoma

<table>
<thead>
<tr>
<th>Study</th>
<th>Cladribine And Other Agent Doses</th>
<th>Other Agents</th>
<th>Histology (%)</th>
<th>Prior Rx</th>
<th>OR %</th>
<th>CR %</th>
<th>Median Duration (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saven et al 1996</td>
<td>A mitoxantrone 57 43</td>
<td>Follic 100</td>
<td>Non 70</td>
<td>70</td>
<td>22</td>
<td>5 (PR)</td>
<td>15 (CR)</td>
</tr>
<tr>
<td>Laurencet et al 1999</td>
<td>B cyclophosphamide prednisone 0 100</td>
<td>42 88</td>
<td>22</td>
<td>12(PR)</td>
<td>15</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Robak et al 1999</td>
<td>C mitoxantrone dexamethasone 0 100</td>
<td>100</td>
<td>29 7</td>
<td>&gt;6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rummel et al 2002</td>
<td>D mitoxantrone 51 49†</td>
<td>32 90</td>
<td>44</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van den Neste et al 2004</td>
<td>E cyclophosphamide 26 74</td>
<td>100</td>
<td>51 14</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Robak et al 2004</td>
<td>F rituximab 12 88‡</td>
<td>100</td>
<td>69 15</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Robak et al 2006</td>
<td>G rituximab cyclophosphamide 0 100††</td>
<td>100</td>
<td>71 11</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laurencet et al 2007</td>
<td>H cyclophosphamide prednisone 0 100‡‡</td>
<td>61 75</td>
<td>30</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inwards et al 2008</td>
<td>I rituximab 0 100‡‡</td>
<td>100</td>
<td>66 52</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† 63% MCL; ††17% MCL; ‡ 65% CLL/SLL; ‡‡100% MCL

CR: complete response; OR overall response

A: 0.1 mg/kg/day as continuous IV infusion for 7 days + mitoxantrone 5 mg/m² IV day 1

B: 0.1 mg/kg/day SC bolus for 3 days; Prednisone 40 mg/m² PO for 5 days; Cyclophosphamide 500 mg/m² IV day 1

C: 0.12 mg/kg/day as 2-h IV infusion for 5 consecutive days + Mitoxantrone 10 mg/m² + Dexamethasone 20 mg/day IV/PO for 5 days

D: 5 mg/m2 2-h IV infusion for 3 consecutive days + Mitoxantrone 8 mg/m2 day 1,2 or 12 mg/m2 day 1 for first relapse

E: 5.6 mg/m²/day for 3 days + Cyclophosphamide 200 mg/m²/day for 3 days

F: 0.12 mg/kg/day as 2-h IV infusion for 5 consecutive days, day 2-6 + Rituximab 375 mg/m2 as 6-h IV infusion day 1
G: 0.12 mg/kg/day as 2-h IV infusion for 3 consecutive days, day 2-4 + Rituximab 375 mg/m² as 6-h IV infusion day 1 + Cyclophosphamide 250 mg/m² day 2-4

H: 0.1 mg/kg/day SC bolus injection for 5 days + Prednisone 40 mg/m² PO for 5 days + Cyclophosphamide 500 mg/m² IV day 1

I: 5 mg/m² 2h IV infusion 5 consecutive days + Rituximab 375 mg/m² 6-h IV infusion day 1
Table 3. Phase II studies of single agent cladribine in previously untreated indolent non-Hodgkin lymphoma

<table>
<thead>
<tr>
<th>Study</th>
<th>Cladribine Doses</th>
<th>Cladribine Histology (%)</th>
<th>Response (%)</th>
<th>Median Duration (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saven et al 1995</td>
<td>A</td>
<td>Follicular: 36, Other: 64</td>
<td>OR: 88, CR: 35</td>
<td>10</td>
</tr>
<tr>
<td>Betticher et al 1996</td>
<td>B</td>
<td>Follicular: 100, Other: 0</td>
<td>OR: 85, CR: 24</td>
<td>&gt;14</td>
</tr>
<tr>
<td>Canfield et al 1997</td>
<td>A</td>
<td>Follicular: 92, Other: 8</td>
<td>OR: 64, CR: 8</td>
<td>&gt;14</td>
</tr>
<tr>
<td>Fridrik et al 1998</td>
<td>C</td>
<td>Follicular: 57, Other: 43</td>
<td>OR: 88, CR: 27</td>
<td>21</td>
</tr>
<tr>
<td>Liliemark et al 1998</td>
<td>C</td>
<td>Follicular: 50, Other: 50</td>
<td>OR: 64, CR: 25</td>
<td>7</td>
</tr>
<tr>
<td>Rummel et al 1999</td>
<td>D</td>
<td>Follicular: 39, Other: 61</td>
<td>OR: 76, CR: 38</td>
<td>23</td>
</tr>
<tr>
<td>Blum et al 2006</td>
<td>E</td>
<td>Follicular: 74, Other: 26</td>
<td>OR: 98, CR: 32</td>
<td>23</td>
</tr>
</tbody>
</table>

CR: complete response; OR overall response

A: 0.1 mg/kg/day as continuous IV infusion for 7 days
B: 0.1 mg/kg/day as continuous IV or SC infusion for 7 days
C: 0.12 mg/kg/day as 2-h IV infusion for 5 consecutive days
D: 5 mg/m² 2-h IV infusion for 5 consecutive days
E: 0.14 mg/kg/day as 2-h IV infusion for 5 consecutive day
Table 4. Phase II studies of single agent cladribine in non-follicular type non-Hodgkin lymphoma

<table>
<thead>
<tr>
<th>Study</th>
<th>Cladribine Doses</th>
<th>Histology</th>
<th>Previously treated</th>
<th>Treatment Naïve</th>
<th>Response (%)</th>
<th>Median Duration (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimopoulos et al 1993&lt;sup&gt;57&lt;/sup&gt;</td>
<td>A 0.1 mg/kg/day as continuous IV infusion for 7 days</td>
<td>WM</td>
<td>20</td>
<td>9</td>
<td>59</td>
<td>3 &gt;9</td>
</tr>
<tr>
<td>Dimopoulos et al 1994&lt;sup&gt;56&lt;/sup&gt;</td>
<td>A 0.12 mg/kg/day as 2-h IV infusion for 5 consecutive days</td>
<td>WM</td>
<td>0</td>
<td>26</td>
<td>85</td>
<td>12 &gt;13</td>
</tr>
<tr>
<td>Fridrik et al 1997&lt;sup&gt;58&lt;/sup&gt;</td>
<td>B 0.14 mg/kg/day as 2-h IV infusion for 5 consecutive days</td>
<td>WM</td>
<td>10</td>
<td>0</td>
<td>90</td>
<td>10 &gt;13</td>
</tr>
<tr>
<td>Liu et al 1998&lt;sup&gt;59&lt;/sup&gt;</td>
<td>B 0.14 mg/kg/day as 2-h IV infusion for 5 consecutive days</td>
<td>WM</td>
<td>13</td>
<td>7</td>
<td>55</td>
<td>5 &gt;18</td>
</tr>
<tr>
<td>Hellman 1999&lt;sup&gt;60&lt;/sup&gt;</td>
<td>C 0.14 mg/kg/day as 2-h IV infusion for 5 consecutive days</td>
<td>WM</td>
<td>13</td>
<td>9</td>
<td>41</td>
<td>36 12 (mean)</td>
</tr>
<tr>
<td>Lefrere et al 2000&lt;sup&gt;55&lt;/sup&gt;</td>
<td>A 0.1 mg/kg/day as continuous IV infusion for 7 days</td>
<td>Splenic MZL</td>
<td>6</td>
<td>1</td>
<td>86</td>
<td>29 4</td>
</tr>
<tr>
<td>Jager et al 2002&lt;sup&gt;54&lt;/sup&gt;</td>
<td>B 0.12 mg/kg/day as 2-h IV infusion for 5 consecutive days</td>
<td>MZL</td>
<td>0</td>
<td>26</td>
<td>100</td>
<td>84 &gt;32</td>
</tr>
<tr>
<td>Rummel et al 1999&lt;sup&gt;53&lt;/sup&gt;</td>
<td>D 0.1 mg/kg/day as continuous IV infusion for 7 days</td>
<td>Mantle Cell</td>
<td>5</td>
<td>7</td>
<td>58</td>
<td>25 19</td>
</tr>
<tr>
<td>Inwards et al 2008&lt;sup&gt;45&lt;/sup&gt;</td>
<td>D 0.12 mg/kg/day as 2-h IV infusion for 5 consecutive days</td>
<td>Mantle Cell</td>
<td>24</td>
<td>26</td>
<td>PT 46, TN 81</td>
<td>PT 21, TN 42, PT:5 14</td>
</tr>
</tbody>
</table>

CR: complete response; OR overall response; MZL: marginal zone lymphoma; PT: previously treated, TN: Treatment naïve.

A: 0.1 mg/kg/day as continuous IV infusion for 7 days
B: 0.12 mg/kg/day as 2-h IV infusion for 5 consecutive days
C: 0.14 mg/kg/day as 2-h IV infusion for 5 consecutive days
D: 5 mg/m² 2-h IV infusion for 5 consecutive days
### Table 5. Phase III study of cladribine with and without cyclophosphamide versus CVP

<table>
<thead>
<tr>
<th>Study</th>
<th>Doses</th>
<th>Histology (%)</th>
<th>Response (%)</th>
<th>3 Year PFS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kalinka et al 2008&lt;sup&gt;61&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Follicular</td>
<td>Non OR</td>
<td>CR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28</td>
<td>72</td>
<td>64 p=0.002</td>
</tr>
<tr>
<td></td>
<td>CdA</td>
<td>24</td>
<td>76</td>
<td>64 p&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>CCdA</td>
<td>37</td>
<td>63</td>
<td>76 p&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>CVP</td>
<td>22</td>
<td>78</td>
<td>44</td>
</tr>
</tbody>
</table>

CR: complete response; OR overall response

CdA = cladribine at 0.12 mg/kg/day as 2-h intravenous infusion for 5 consecutive days

CCdA = same cladribine plus cyclophosphamide 800 mg/m<sup>2</sup> bolus IV every 28 days

CVP = cyclophosphamide 800 mg/m<sup>2</sup> bolus IV day 1, vincristine 1.4 mg/m<sup>2</sup> bolus IV day 1; prednisone 45 mg/m<sup>2</sup> orally days 1 to 4 every 21 days
Table 6. Phase II CLL cladribine studies – previously treated patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of Patients</th>
<th>Prior Therapies (median)</th>
<th>Cladribine Doses and Other Agents</th>
<th>Response (%) OR</th>
<th>CR</th>
<th>Median Duration (months)</th>
</tr>
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<tbody>
<tr>
<td>Saven et al 1991</td>
<td>90</td>
<td>2</td>
<td>A, B</td>
<td>44</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Tallman et al 1995</td>
<td>26</td>
<td>2</td>
<td>A</td>
<td>31</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Robak et al 1996</td>
<td>92</td>
<td>2</td>
<td>C</td>
<td>36</td>
<td>5</td>
<td>NA</td>
</tr>
<tr>
<td>JULIUSSON et al 1996</td>
<td>52</td>
<td>2</td>
<td>C</td>
<td>58</td>
<td>31</td>
<td>20</td>
</tr>
<tr>
<td>Rondelli et al 1999</td>
<td>19</td>
<td>&gt;2</td>
<td>D</td>
<td>68</td>
<td>10</td>
<td>CR, 9+</td>
</tr>
<tr>
<td>Robak et al 2000</td>
<td>104</td>
<td>2</td>
<td>E</td>
<td>51</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>2</td>
<td>C</td>
<td>45</td>
<td>9</td>
<td>9</td>
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<tr>
<td>ROBAK et al 2001</td>
<td>19</td>
<td>2</td>
<td>F, G</td>
<td>37</td>
<td>5</td>
<td>NA</td>
</tr>
<tr>
<td>Karlsson et al 2002</td>
<td>38</td>
<td>2</td>
<td>H</td>
<td>34</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Montillo et al 2003</td>
<td>20</td>
<td>2</td>
<td>I</td>
<td>35</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Robak et al 2004</td>
<td>15</td>
<td>2</td>
<td>J</td>
<td>73</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>

NA = not available
A = 0.1 mg/kg/day as continuous IV infusion for 7 days
B = 0.028-0.14 mg/kg/day as 2-h IV infusion for 5 consecutive days.
C = 0.12 mg/kg/day d1-5 2-h intravenous bolus
D = 6 mg/m²/day as 2-h IV infusion for 5 consecutive days
E = 0.12 mg/kg/d 2h IV infusion for 5 consecutive days + Prednisone 30 mg/m² PO for 5 days
F = 0.12 mg/kg/day as 2-h IV infusion for 5 consecutive days + Cyclophosphamide 650 mg/m² IV day 1 + Mitoxantrone 10 mg/m² IV day 1
G = 0.12 mg/kg/day as 2-h IV infusion for 3 consecutive days + Cyclophosphamide 650 mg/m² IV on day 1 + Mitoxantrone 10 mg/m² IV on day 1
H = 10 mg/m²/day PO for 3 consecutive days q21 days
I = 4 mg/m²/day as 2-h IV infusion for 3 consecutive days + Cyclophosphamide 350 mg/m²/d IV for 3 days
J = 0.12 mg/kg/day as 2-h IV infusion for 5 consecutive days, day 2-6 + Rituximab 375 mg/m² as 6-h IV infusion day 1
Table 7. Phase III CLL cladribine studies – untreated patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Regimen</th>
<th>Number of Patients</th>
<th>Cladribine Dose</th>
<th>Response (%)</th>
<th>Median Duration (months)</th>
<th>OS (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robak et al 2000&lt;sup&gt;87&lt;/sup&gt;</td>
<td>2-CdA + P</td>
<td>126</td>
<td>A</td>
<td>87</td>
<td>At 24 months: 46%</td>
<td>At 24 months: 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47</td>
<td>p-value= 0.01</td>
<td>p-value= 0.6</td>
</tr>
<tr>
<td></td>
<td>Chl + P</td>
<td>103</td>
<td>12 mg/m²/d PO + 30 mg/m²/d PO for 7 days</td>
<td>57</td>
<td>At 24 months: 33%</td>
<td>At 24 months: 78%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
<td>p-value= 0.6</td>
<td></td>
</tr>
<tr>
<td>Robak et al 2006&lt;sup&gt;88&lt;/sup&gt;</td>
<td>2-CdA</td>
<td>166</td>
<td>B</td>
<td>77</td>
<td>24</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21</td>
<td>p-value= 0.47</td>
<td>p-value= 0.73</td>
</tr>
<tr>
<td></td>
<td>2-CdA + Cy</td>
<td>162</td>
<td>C</td>
<td>83</td>
<td>22</td>
<td>Not yet reached</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>29</td>
<td>p-value= 0.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-CdA + Cy + M</td>
<td>151</td>
<td>D</td>
<td>80</td>
<td>24</td>
<td>Not yet reached</td>
</tr>
<tr>
<td>Karlsson et al 2007&lt;sup&gt;86&lt;/sup&gt;</td>
<td>2-CdA</td>
<td>72</td>
<td>E</td>
<td>75 NA</td>
<td>25</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p-value= 0.0003</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>73</td>
<td>25 mg/m²/d IV for 5 days</td>
<td>70</td>
<td>10</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25 mg/m²/d</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chl</td>
<td>76</td>
<td>10 mg/m²/d PO for 10 days</td>
<td>62</td>
<td>9</td>
<td>62</td>
</tr>
<tr>
<td>Robak et al 2010&lt;sup&gt;89&lt;/sup&gt;</td>
<td>2-CdA + Cy</td>
<td>212</td>
<td>F</td>
<td>88</td>
<td>28</td>
<td>At 48 months 62%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47</td>
<td>p-value= 0.51</td>
<td>p-value= 0.16</td>
</tr>
<tr>
<td></td>
<td>F + Cy</td>
<td>211</td>
<td>25 mg/m²/d IV + 250 mg/m²/d IV for 3 days</td>
<td>82</td>
<td>61</td>
<td>At 48 months 61%</td>
</tr>
</tbody>
</table>

2-CdA, cladribine; F, fludarabine; P, prednisone; Chl, chlorambucil; Cy, cyclophosphamide; M, mitoxantrone; PO, oral; IV, intravenous; NS, not significant

A = 0.12 mg/kg/day 2h IV infusion for 5 consecutive days + Prednisone 30 mg/m² PO for 5 days

B = 0.12 mg/kg/day as 2-h IV infusion for 5 consecutive days
C = 0.12 mg/kg/day as 2-h IV infusion for 3 consecutive days + Cyclophosphamide 650 mg/m² IV on day 1
D = 0.12 mg/kg/day as 2-h IV infusion for 3 consecutive days + Cyclophosphamide 650 mg/m² IV on day 1 + Mitoxantrone 10 mg/m² IV on day 1
E = 5 mg/m² 2-h IV infusion for 5 consecutive days
F = 0.12 mg/kg/day as 2-h IV infusion for 3 consecutive days + Cyclophosphamide 250 mg/m²/day IV for 3 consecutive days
Table 8. Cladribine compared to fludarabine in CLL

<table>
<thead>
<tr>
<th>Study</th>
<th>Regimen</th>
<th>Number of Patients</th>
<th>Response (%)</th>
<th>Median Duration (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robak et al 2006&lt;sup&gt;88&lt;/sup&gt;</td>
<td>2-CdA + Cy</td>
<td>162</td>
<td>77, 21</td>
<td>24</td>
</tr>
<tr>
<td>Eichhorst et al 2006&lt;sup&gt;94&lt;/sup&gt;</td>
<td>F + Cy</td>
<td>180</td>
<td>94, 24</td>
<td>48</td>
</tr>
<tr>
<td>Catovsky et al 2007&lt;sup&gt;93&lt;/sup&gt;</td>
<td>F + Cy</td>
<td>196</td>
<td>94, 38</td>
<td>43</td>
</tr>
<tr>
<td>Flinn et al 2007&lt;sup&gt;67&lt;/sup&gt;</td>
<td>F + Cy</td>
<td>141</td>
<td>74, 23</td>
<td>32</td>
</tr>
<tr>
<td>Robak et al 2010&lt;sup&gt;39&lt;/sup&gt;</td>
<td>2-CdA + Cy</td>
<td>211</td>
<td>88, 47</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>F + Cy</td>
<td>212</td>
<td>82, 46</td>
<td>27</td>
</tr>
</tbody>
</table>

2-CdA, cladribine; F, fludarabine; Cy, cyclophosphamide
Table 9. Cladribine and fludarabine toxicities

<table>
<thead>
<tr>
<th>Study</th>
<th>Regimen</th>
<th>Phase</th>
<th>Disease</th>
<th>Grade 3/4 Neutropenia (%)</th>
<th>Grade 3/4 Thrombocytopenia (%)</th>
<th>Grade 3/4 Infections (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roback et al 2000&lt;sup&gt;87&lt;/sup&gt;</td>
<td>2-CdA + P</td>
<td>III</td>
<td>CLL</td>
<td>9</td>
<td>9</td>
<td>56 (all grades)</td>
</tr>
<tr>
<td>Robak et al 2004&lt;sup&gt;75&lt;/sup&gt;</td>
<td>2-CdA + R</td>
<td>II</td>
<td>CLL &amp; NHL (previously treated)</td>
<td>12</td>
<td>13</td>
<td>27</td>
</tr>
<tr>
<td>Karlsson et al 2007&lt;sup&gt;86&lt;/sup&gt;</td>
<td>2-CdA</td>
<td>III</td>
<td>CLL</td>
<td>57</td>
<td>43</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>III</td>
<td>CLL</td>
<td>34</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Robak et al 2010&lt;sup&gt;89&lt;/sup&gt;</td>
<td>2-CdA + Cy</td>
<td>III</td>
<td>CLL</td>
<td>20</td>
<td>12</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>F + Cy</td>
<td>III</td>
<td>CLL</td>
<td>21</td>
<td>11</td>
<td>27</td>
</tr>
</tbody>
</table>

2-CdA, cladribine; F, fludarabine; P, prednisone; Cy, cyclophosphamide; R, rituximab
Figure Legends

Figure 1. Molecular structures of 2’-deoxyadenosine and cladribine.

Cladribine has a chlorine atom substituted at the 2-position of the purine ring.

(Illustration: Katya Kadyshevskaya)

Figure 2. Cladribine mechanism of action. Cladribine (2-CdA) enters the cell through an efficient transport system. Cladribine is resistant to deamination by adenosine deaminase (ADA). The high deoxycytidine kinase (DCK) to 5’-nucleotidase (5’NT) ratio favors the formation of 2-chlorodeoxyadenosine monophosphate (2-CdAMP), -diphosphate (2-CdADP), and triphosphate (2-CdATP). In dividing cells the accumulation of 2-CdTP inhibits ribonucleotide reductase (RNR) and the DNA polymerases (DNAP), abrogating DNA synthesis and causing cell death. In resting cells, 2 unique pathways result in apoptosis. First, cladribine increases DNA strand breaks, activating a poly(ADP-ribose)polymerase which depletes NAD and ATP, resulting in apoptosis. Second, cladribine alters the mitochondrial membrane resulting in cytoplasmic translocation of cytochrome c and nuclear translocation of apoptosis-inducing-factor (AIF), which lead to caspase-dependent and -independent apoptosis, respectively. Hypomethylation may occur through cladribine’s inhibition of S-adenosylhomocysteine thereby diminishing the methyl donor pool.9 (Illustration: Katya Kadyshevskaya)
Figure 1

2'DEoxyADenosine

CLAdribine
Beyond hairy cell: the activity of cladribine in other hematologic malignancies

Darren S. Sigal, Heather J. Miller, Ethan D. Schram and Alan Saven