Purine Analogs for the Treatment of Low-Grade Lymphoproliferative Disorders

PANOS FIDIAS, BRUCE A. CHABNER, MICHAEL L. GROSSBARD

Hematology-Oncology Unit, Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts, USA

Key Words. Hairy cell leukemia · Lymphoma non-Hodgkin’s · Leukemia chronic lymphocytic · Pentostatin · Cladribine · Vidarabine analogs

Abstract

Primary purpose. Low-grade lymphoproliferative disorders follow an indolent clinical course but are incurable with current therapy. Recently, three active agents for the treatment of these diseases have been identified: the purine analogs fludarabine, pentostatin and 2-chlorodeoxyadenosine. The purpose of this review is to summarize the current knowledge on the mechanism of action, clinical activity and toxicities of the purine analogs.

Methods. Articles, abstracts and letters to the editor appearing in English literature and involving the use of the purine analogs in the treatment of hairy cell leukemia, chronic lymphocytic leukemia, indolent non-Hodgkin’s lymphoma, cutaneous T cell lymphomas and Waldenström’s macroglobulinemia were reviewed.

Results and conclusion. Purine analogs have marked cytoreductive potential in the treatment of chronic lymphocytic leukemia, indolent non-Hodgkin’s lymphoma and hairy cell leukemia. Major side effects include myelosuppression and infections. Profound lymphocytopenia can be sustained, predisposing patients to opportunistic infections. Although remissions achieved with these agents can be long-lasting, minimal residual disease frequently persists. Postremission strategies aimed at eradicating such microscopic diseases can potentially improve the results of purine analog therapy. Alternatively, the up-front combination of these agents with traditional chemotherapy may lead to higher response rates and more sustained remissions.

The Oncologist 1996;1:125-139

Introduction

Low-grade lymphoproliferative disorders affect more than 40,000 Americans annually. Although disease in the majority of patients will follow an indolent course, the ultimate development of symptoms will dictate the use of cytotoxic treatment. With the exception of hairy cell leukemia (HCL), therapy for these low-grade malignancies is based on alkylating agents, with or without prednisone. The addition of other agents has added to the toxicities of therapy without convincingly increasing the survival rates [1-3].

In order to improve the outlook of patients with low-grade lymphoid malignancies effective new agents are needed. The purine analogs 2-chloro-2′-deoxyadenosine (2-CdA or cladribine), 9-β-D-arabinofuranosyl-2-fluoroadoenosine-5′-monophosphate (fludarabine), and 2′-deoxycoformycin (DCF or pentostatin) (Fig. 1) have demonstrated marked activity against resting lymphocytes. Early clinical trials established the remarkable efficacy of pentostatin and 2-CdA against HCL [4] and stimulated a strong interest in the aggressive management of lymphoid malignancies that previously were approached with palliative intent. This article reviews the mechanism of action of the purine analogs and provides a summary of the clinical trials conducted with these agents.

Purine Metabolism and Inherited ADA Deficiency

In 1972, Giblett showed that some children with severe combined immunodeficiency (SCID) had an inherited deficiency of the enzyme adenosine deaminase (ADA) [5]. ADA is widely distributed, but the highest concentrations are seen in the thymus, spleen and circulating lymphocytes [6, 7]. The main function of ADA is to regulate the concentration of intracellular deoxyadenosine (dAdo) (Fig. 1). Deoxadenosine triphosphate (dATP) (Fig. 2) is a toxic metabolite, and its accumulation directly correlates with its lymphocytolytic effect [8]. This selective toxicity for lymphoid cells is explained by the high levels of phosphorylating enzymes (deoxycytidine kinase or dCK) present in both proliferating and resting lymphocytes,
which are capable of producing toxic concentrations of dATP when the catabolic pathway of dAdo is blocked [9]. Therefore, it is logical to expect that pharmacologic inhibition of ADA or high concentrations of dAdo analogs resistant to ADA deamination would result in lymphotoxicity.

**MECHANISM OF ACTION**

**Pentostatin**

Although pentostatin lacked antitumor activity in preclinical screening, it was developed initially as a companion to Ara-A (vidarabine) because of its extreme potency in inhibiting ADA, the enzyme that deaminates Ara-A and limits its clinical activity [8]. By virtue of its structural similarities to the reaction intermediates during the deamination of dAdo, pentostatin acts in a pseudo-irreversible manner as a tight-binding inhibitor of ADA with a very slow dissociation rate from the enzyme (T1/2 ~ 68 h) [10].

The main intracellular effect of ADA inhibition is the accumulation of dAdo and dATP, which can lead to lethal base substitutions and defective DNA repair. dAdo also can interfere with cellular metabolism by inhibiting S-adenosylhomocysteine hydrolase and disrupting important methylation reactions requiring S-adenosylmethionine (SAM) [8]. Although increased levels of SAM have been detected in patients with ADA deficiency and in red cells and lymphoblasts of a patient on pentostatin [11, 12], not all investigators could correlate cytotoxicity with S-adenosylhomocysteine hydrolase inhibition [13, 14].

**Fludarabine**

Fludarabine (F-Ara-A) was synthesized as the ADA resistant analog of Ara-A, but clinical use of the compound was restricted by its relative insolubility. This difficulty was circumvented by the addition of a phosphate group (fludarabine monophosphate). F-Ara-A is taken up by cells through the nucleoside transporter mechanism and is phosphorylated intracellularly to fludarabine triphosphate (F-Ara-ATP). The rate limiting enzyme for this phosphorylation is dCK [15]; hence, fludarabine accumulates mainly in cells with high activity of this enzyme, such as lymphocytes.

F-Ara-ATP is a potent inhibitor of DNA polymerase, mainly polymerase-α [16], and ribonucleotide reductase [17]. Other important enzymes affected by fludarabine are DNA primase [18] and DNA ligase I [19]. In its monophosphate form, fludarabine is incorporated into DNA, causing premature termination of chain elongation [16]. F-Ara-A also is incorporated into RNA [20].

**2-Chlorodeoxyadenosine**

2-CdA was the most active ADA-resistant nucleoside analog tested against various cell lines [15]. Within cells, 2-CdA is phosphorylated by dCK in a fashion similar to fludarabine, but the triphosphate forms accumulate to a much lesser degree than F-Ara-ATP. 2-CdA, like fludarabine, can effectively inhibit RNR and DNA polymerase [17, 21]. 2-CdA also is incorporated into DNA, producing strand breaks and inhibition of DNA synthesis [22], but it does not appear to be as potent a chain terminator as fludarabine [23].

Inhibition of RNR or DNA polymerase is expected to affect actively proliferating cells. In resting cells, however, the activity of these enzymes is virtually undetectable and other mechanisms must be responsible for cytotoxicity.

**Figure 1. The structures of deoxyadenosine (A), and the purine analogs fludarabine (B), 2-chlorodeoxyadenosine (C), and 2′-deoxycoformycin (D).**

**Figure 2. Purine metabolism.** RNR: Ribonucleotide reductase; AMPDA: Adenosine monophosphate deaminase; dCK: Deoxycytidinekinase; ADA: Adenosine deaminase; PNP: Purine nucleoside phosphorylase. Other enzymes: Adenine phosphoribosyl-transferase (1); Hypoxanthine-guanine phosphoribosyltransferase (2); 5′-Nucleotidase (3); Adenylosuccinate synthetase (4).
Nondividing lymphocytes continuously undergo some degree of DNA repair, mediated in part through the modification of nuclear proteins by polyADP-ribosylation [24, 25]. This process is associated with an expenditure of cellular NAD (nucleoside adenine phosphate) and ATP [21]. Possibly, by interfering with DNA repair, the purine analogs accelerate polyADP-ribose formation and may induce lethal NAD and ATP depletion. This theory is in accord with findings that correlate the toxicity and therapeutic efficacy of pentostatin with the ratio of dATP/ATP [26, 27].

Although the exact mechanism of action of the purine analogs is unclear, it has been suggested that the common final pathway of their lymphotoxicity is the induction of DNA fragmentation. Recent reports demonstrate that the exposure of normal and malignant lymphocytes to fludarabine and 2-CdA triggers degradation of DNA into nucleosomal-sized multimers, which is the hallmark of apoptosis [28, 29].

**Clinical Activity of the Purine Analog**

**Hairy Cell Leukemia**

**Pentostatin**

Pentostatin revolutionized the management of HCL. Previously established therapies, such as splenectomy and interferon, could improve the hematologic parameters, but only rarely could eradicate leukemic cells from the bone marrow [30]. In contrast, 33%-89% of patients treated with pentostatin achieve a complete response after 2-3 cycles of chemotherapy (Table 1) [31-37]. Within 24-48 h, there is a marked reduction in the number of circulating hairy cells, occasionally accompanied by fever [38]. The platelet count increases within one to two weeks, while the neutrophils and red cells require several weeks to recover. Splenomegaly regresses within a week, although several courses may be necessary for larger spleens to enter remission [33]. A recent randomized study compared pentostatin to interferon-α (3 × 10^6 IU three times weekly for a total of six months) in 313 patients. The results showed a higher complete remission rate with pentostatin compared to interferon-α.

### Table 1. Purine nucleoside analogs in hairy cell leukemia

<table>
<thead>
<tr>
<th>Author</th>
<th>Schedule</th>
<th>Patients (#)</th>
<th>Untreated (#)</th>
<th>RR (CR%)</th>
<th>Follow-up (months)</th>
<th>Relapse (#)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DCF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Johnston [31]</td>
<td>4 mg/m² qw × 3 q8w</td>
<td>28</td>
<td>18</td>
<td>100 (89)</td>
<td>14.3</td>
<td>1/28</td>
</tr>
<tr>
<td>Ho [32]</td>
<td>4 mg/m² qw × 3; then qw × 6w</td>
<td>33</td>
<td>0</td>
<td>79 (33)</td>
<td>14.5</td>
<td>2/26</td>
</tr>
<tr>
<td>Kraut [33]</td>
<td>4 mg/m² qow</td>
<td>23</td>
<td>10</td>
<td>96 (87)</td>
<td>2-24</td>
<td>5/22</td>
</tr>
<tr>
<td>Cassileth [34]</td>
<td>5 mg/m²/d × 2d qow</td>
<td>50</td>
<td>19</td>
<td>84 (64)</td>
<td>&gt;36</td>
<td>6/42</td>
</tr>
<tr>
<td>Golomb [35]</td>
<td>4 mg/m² qow</td>
<td>85</td>
<td>0</td>
<td>83 (42)</td>
<td>44</td>
<td>12/69</td>
</tr>
<tr>
<td>Catovery [36]</td>
<td>4 mg/m² qow</td>
<td>156</td>
<td>23</td>
<td>95 (70)</td>
<td>NS</td>
<td>12/147</td>
</tr>
<tr>
<td>Grever [37]</td>
<td>4 mg/m² qow</td>
<td>154</td>
<td>154</td>
<td>79 (76)</td>
<td>57</td>
<td>10/117 CR</td>
</tr>
<tr>
<td><strong>2-CdA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hoffman [41]</td>
<td>0.1 mg/kg/d × 7d CIV</td>
<td>11</td>
<td>6</td>
<td>100 (82)</td>
<td>5.5</td>
<td>NS</td>
</tr>
<tr>
<td>Piro [42]</td>
<td>0.1 mg/kg/d × 7d CIV</td>
<td>148</td>
<td>69</td>
<td>97 (85)</td>
<td>14</td>
<td>2/44</td>
</tr>
<tr>
<td>Estey [43]</td>
<td>4 mg/m²/d × 7d CIV</td>
<td>46</td>
<td>27</td>
<td>89 (78)</td>
<td>9.2</td>
<td>2/42</td>
</tr>
<tr>
<td>Juliusson [44]</td>
<td>0.1 mg/kg/d × 7d CIV</td>
<td>16</td>
<td>3</td>
<td>75 (75)</td>
<td>12</td>
<td>0/12</td>
</tr>
<tr>
<td>Tallman [45]</td>
<td>0.1 mg/kg/d × 7d CIV</td>
<td>20</td>
<td>15</td>
<td>100 (90)</td>
<td>12</td>
<td>1/20</td>
</tr>
<tr>
<td>Lauria [46]</td>
<td>0.1 mg/kg/d × 7d CIV</td>
<td>37</td>
<td>12</td>
<td>100 (78)</td>
<td>NS</td>
<td>4/37</td>
</tr>
<tr>
<td>Filleul [47]</td>
<td>0.1 mg/kg/d × 7d CIV</td>
<td>10</td>
<td>1</td>
<td>90 (80)</td>
<td>12</td>
<td>1/8 CR</td>
</tr>
<tr>
<td>Juliusson [48]</td>
<td>3.4 mg/m²/d × 7d SC</td>
<td>73</td>
<td>37</td>
<td>96 (81)</td>
<td>20</td>
<td>0/70</td>
</tr>
<tr>
<td><strong>Fludarabine</strong></td>
<td>30 mg/m²/d × 5d q month</td>
<td>3</td>
<td>0</td>
<td>67 (0)</td>
<td>13-18</td>
<td>1/2</td>
</tr>
</tbody>
</table>

RR: Response Rate; CR: Complete Response; CIV: Continuous i.v. infusion; SC: Subcutaneous; NS: Not Stated.

*One patient received 2 mg/m² and one patient received 3 mg/m².

*Few patients received 5 mg/m²/d × 3d q4w.

*Patients with performance status 3-4 received 2 mg/m².

*The first 40 patients received 4 mg/m² qw × 4, and then qw.

*Total of 29 patients, with 28 patients eligible.

*Total of 26 patients, with 20 patients eligible.

*Total of 159 patients, with 156 patients eligible.

*Patients achieved response with a single course of 2-CdA, except in the studies by Tallman (2/20 responders required a second course), Filleul (1/9 responders required a second course), and Juliusson (5/70 responders required a second course of s.c. 2-CdA).
previously untreated patients [37]. Significant differences in both overall response rate (79% versus 38%) and disease-free survival at a median of 57 months were noted in favor of pentostatin (107/117 complete responders on pentostatin remained in remission versus 5/17 complete responders on interferon-alone). Although no overall survival advantage was seen, the crossover design of the study complicated this assessment. One hundred and four patients failing interferon were crossed over to pentostatin, and 75% of these patients responded.

The remissions achieved with pentostatin appear durable since only approximately 10% of the patients relapse after 2-57 months of follow-up [31-37]. It is unclear, however, if pentostatin is a curative treatment. Thaler et al. [39] showed that residual leukemia could be detected with immunoperoxidase staining of the bone marrow in six patients following pentostatin treatment with nadir hairy cell numbers of 0.2%-3.0% of total bone marrow cells. In a recent report [40] on the long-term follow-up (median 82 months) of patients achieving complete remission with pentostatin, 11 of 23 patients relapsed, and seven of these patients have required retreatment at a median of 60 months from initial remission.

2-CdA

Many clinical trials have confirmed the unique sensitivity of HCL to a single course of 2-CdA [41-48]. Complete eradication of the disease is seen in 75%-90% of patients, and the overall response rate (RR) is 10%-75%. Only eight out of 345 responding patients reported in the literature required a second course to enter remission (Table 1). The pattern of response was similar to that seen with pentostatin. The total leukocyte count decreased rapidly during the infusion, with a concomitant dramatic decline in the number of circulating hairy cells; platelets normalized first, followed by neutrophil and hemoglobin recovery within eight weeks.

Although responses after 2-CdA treatment appear durable, the follow-up has been short (Table 1). Minimal residual disease can be detected in a portion of patients in morphologic remission. Hakimian et al. [49] used immunostaining of bone marrow specimens to identify residual hairy cells in five out of 24 complete responders. Tallman et al. [45] used peripheral blood flow cytometry and/or immunoglobulin gene rearrangement studies to assess clonality in 13 complete responders, and found two patients with evidence of residual disease. The clinical significance of these findings is uncertain.

Fludarabine

Experience with fludarabine in the treatment of HCL is limited [50, 51]. Kantarjian et al. [51] reported on three patients previously treated with either interferon alone, splenectomy and interferon, or splenectomy, interferon and pentostatin. Two patients had partial responses, lasting 13+ and 18 months, while the patient previously treated with pentostatin had a minor response. The role of fludarabine in the treatment of HCL is not well established and, since the results with the other nucleoside analogs are so impressive, it may never be defined.

Chronic Lymphocytic Leukemia

Fludarabine

The use of fludarabine in chronic lymphocytic leukemia (CLL) is associated with objective responses in 70%-79% of untreated patients and in 12%-57% of previously treated patients [52-65] (Table 2). Clinical improvement usually is rapid, with the majority of responders obtaining their maximum benefit within three cycles. Remission duration after fludarabine treatment ranges from six months to three years, depending on whether the patients were previously treated and whether they achieved a good quality response with fludarabine. In the study by O’Brien et al., the median time to progression for the previously untreated patients who achieved a complete response (CR) with fludarabine was not reached with a median follow-up of two years [59]. The number of prior treatments correlates with response in some [66], but not all studies [53, 55]. Similarly, the pretreatment Rai stage has not been consistently predictive of response [53, 58, 59, 64], and even patients with advanced stage disease can experience significant initial reduction of their tumor burden. However, several parameters indicative of biologically active disease, such as high levels of 2-microglobulin, depressed levels of immunoglobulins and low serum albumin, have been consistently associated with poor RRs and survival [58, 64].

Most commonly, fludarabine is administered as a bolus infusion daily for five days every four weeks at a dose of 25-30 mg/m^2. There is no apparent difference between the 25 mg/m^2 and 30 mg/m^2 regimens [53]. Modifications of this “standard” schedule have not increased the activity of fludarabine [55, 58, 59].

More recently, randomized trials were initiated comparing fludarabine to doxorubicin-containing chemotherapy for patients with CLL. Preliminary results have been reported in two of these trials. Hiddeman et al. [61] not only found a significantly higher response using fludarabine versus cyclophosphamide, doxorubicin, prednisone (CAP) therapy (untreated patients 70% versus 58%, previously treated patients 45% versus 26%), but also noted a higher frequency of bronchopulmonary infections in the fludarabine arm. Binet et al. [62] stratified patients according to stage and treated them with either fludarabine alone, CAP or low-dose cyclophosphamide, doxorubicin, vincristine and prednisone (mini-CHOP).
Fludarabine induced a higher response rate in stage B patients (94% versus 72% versus 75%), but not in stage C patients (64% versus 84% versus 62%). These differences are not statistically significant, and no survival benefit has been seen, albeit with short follow-up. The North American intergroup trial randomized previously untreated patients with active CLL to therapy with fludarabine or chlorambucil. A third arm, which combined the two drugs, was closed due to unacceptably high hematologic toxicity. The patients treated with fludarabine had a similar partial response (PR) rate (38% versus 8%), but a significantly higher CR rate (33% versus 8%) compared to the patients treated with chlorambucil.
Longer follow-up will be necessary to determine whether there is any survival difference between the two arms [65].

Salvage therapy in patients who relapse after fludarabine is not well-established. In a recent report [64] on patients receiving fludarabine as initial therapy for CLL, relapsed patients had a salvage rate of 61% when retreated with fludarabine, compared to 25% when treated with other regimens.

2-CdA

The clinical activity of 2-CdA in CLL is shown in Table 2 (RR 31%-85%, CR 0%-47%). Cladribine can be administered in a variety of schedules and routes, including a continuous i.v. infusion, a daily bolus infusion, s.c. injections, and as a solution for p.o. dosing. Although no randomized trials have been conducted, and the patient populations in the phase II studies are not directly comparable, no apparent response differences can be demonstrated with the various treatment schedules [67-72].

Clinical characteristics such as stage [67] and sensitivity to prior chemotherapy [68] have been shown to predict the likelihood of response to 2-CdA treatment. In an attempt to identify more precise predictors of clinical response, Kawasaki et al. [73] determined the ratio of the pretreatment levels of the key enzymes in the metabolism of the purine analogs dCK and 5′-nucleotidase. When the ratio was high, suggesting that the accumulation of the drug was favored over its catabolism, patients appeared to be more responsive to treatment.

Pentostatin

Table 2 summarizes the clinical activity of pentostatin in CLL. Responses have been demonstrated in B cell and T cell CLL, pro lymphocytic leukemia, large granular lymphocytosis and Sezary cell leukemia [74-79].

Most trials have employed an initial weekly schedule for three to four weeks, followed by infusions every other week until maximum response. Pentostatin yields an RR of 30%-50% in CLL patients, but the CR rate is low, ranging from 0%-11%. Of note, Mericieca et al. [79] found an RR of 45% in 55 patients with T cell prolymphocytic leukemia, a disease characterized by unresponsiveness to conventional chemotherapy and limited life expectancy. The same authors noted a higher activity of pentostatin against CD4+ leukemias, as opposed to CD4- leukemias (RR 56% versus 23%). In another study, Grever et al. [74] attempted to correlate clinical results with the level of ADA inhibition. Despite the fact that pentostatin could ablate ADA activity in all cases tested, 82% of the patients did not respond, suggesting that pathways distal to ADA inhibition must be essential for its cytotoxic effect. Moreover, the pretreatment ADA activity did not differ between responders and nonresponders. Given the activity of fludarabine and 2-CdA in CLL, it appears unlikely that pentostatin will have a major role in the therapy of this disease.

Non-Hodgkin’s Lymphomas

Fludarabine

Fludarabine has preferential activity against low-grade histologies, with approximately half of the patients expected to respond [80-86] (Table 3). In contrast, the RRs for intermediate and high-grade non-Hodgkin’s lymphoma (NHL) range from 0%-7% [80, 82, 83]. Fludarabine has also been used in patients achieving a minimal residual disease state after traditional chemotherapy in an attempt to provide additional cyto-reduction prior to bone marrow transplantation. Pigaditou et al. [86] reported on 20 evaluable patients with an RR of 45%. However, the exact criteria for the detection and follow-up of minimal residual disease were not stated.

Experience with fludarabine in cutaneous lymphomas is limited. Von Hoff et al. [81] administered fludarabine to 31 previously treated patients with mycosis fungoides at a dose of 25 mg/m² for five days for those without prior systemic therapy (good risk), or 18 mg/m² for five days for patients previously treated with cytotoxic therapy (poor risk). Three of ten good risk patients, and 3 of 21 poor risk patients responded.

Previous therapy may limit the activity of fludarabine. Zinzani et al. [84] administered fludarabine (25 mg/m²/day × 5 days every month) to 8 chemotherapy-naive and 13 previously treated NHL patients, and found a CR rate of 37% in the previously untreated population, as compared with no CR in the previously treated patients. Pigaditou et al. [86] used the same schedule in 51 relapsed and 16 previously untreated patients, and showed an RR of 45% for the former group and 69% for the latter group. Nevertheless, prior therapy does not preclude a benefit from fludarabine treatment, even after doxorubicin has failed: 12 out of the 18 responders in the series by Hochster et al. [83], using a relatively low dose of fludarabine (18 mg/m²), had received doxorubicin-containing combination chemotherapy.

2-CdA

Several clinical trials suggest that 2-CdA can induce remissions in 27%-55% of relapsed or refractory indolent NHLs [87-94] (Table 3). A single study performed in 28 chemotherapy-naive patients attained an impressive RR of 89% [95]. The activity of 2-CdA in cutaneous T cell lymphomas (CTCL) was evaluated by Saven et al. [87] in 16 patients relapsing after topical only (seven patients) or topical and systemic (nine patients) therapy. Three of nine patients with mycosis fungoides responded, and four of
seven patients with nonmycosis fungoides’ histologies responded. In contrast, only one of eight patients with mycosis fungoides achieved a remission in the study by O’Brien et al. [92]. Lee et al. [91] noted an RR of 30% in 20 previously treated CTCL patients, but identified no responses in four patients with large cell lymphoma of the skin, either de novo or transformed from a pre-existing low-grade lymphoma.

Pentostatin

Although there has been strong interest in the use of this agent as therapy for CTCL, the clinical reports suffer from small numbers of patients, and their results must be interpreted with caution. Grever et al. [96] administered pentostatin at doses of 4-10 mg/m^2/day for three days every four weeks to four patients with poor prognosis mycosis fungoides as evidenced by extensive plaque disease of skin, skin tumors, lymph node involvement, or hepatosplenomegaly. All patients responded with regression of skin lesions, lymphadenopathy and splenomegaly lasting from four to nine months. In the largest series of CTCL patients [79, 29] those receiving a dose of 4 mg/m^2 weekly for four weeks and then every other week until maximum benefit, demonstrated an RR of 34%, which was unevenly distributed among the different histologic subtypes. All responses were seen in the 16 patients with Sezary syndrome, with no responses observed in the mycosis fungoides group.
In the nodal lymphomas, RRs appear to be inferior to the other nucleoside analogs [79, 97, 98] (Table 3). The Cancer and Leukemia Group B conducted a trial using pentostatin at a dose of 4 mg/m² weekly for three weeks and then every other week in 76 previously treated patients, 49 of whom had noncutaneous low-grade lymphomas [97]. Only eight of 49 patients responded. Using a similar treatment schedule, Mercieca et al. [79] reported an RR of 19% (0% CR) among 27 patients with T cell lymphomas, and 12% (8% CR) among 25 patients with human T cell lymphoma/leukemia virus (HTLV-1) associated adult T cell leukemia/lymphoma.

**Purine Nucleoside Analogs in Waldenström’s Macroglobulinemia (WM)**

Several cases of macroglobulinemic lymphoma have been included in reports of NHL patients treated with purine analogs [84, 85, 89, 90], but only a few studies are restricted to this clinical entity (Table 4). Dimopoulos et al. [99] administered fludarabine to 28 patients with WM, two of whom were previously untreated. Thirty-six percent of the patients responded, with a median response duration of 38 months. The same authors treated 29 WM patients, nine of whom were previously untreated, with 2-CdA for a total of two courses [100]. All previously untreated patients responded, while no patient refractory to prior chemotherapy for more than 12 months responded. Overall, the RR was 59%, with a 4% CR rate. Delannoy et al. [101] reported on the activity of 2-CdA in 13 previously treated and five untreated patients. No CRs were seen, but seven patients achieved a PR, five of whom were previously treated. Dimopoulos et al. [102] administered two courses of 2-CdA therapy to 26 patients without prior chemotherapy exposure, and noted an impressive overall RR of 85% and a CR rate of 12%. With a median follow-up of 13 months, five patients have relapsed, and three out of the four patients requiring treatment responded again to 2-CdA.

One case report [103] describes a patient with refractory Waldenström’s and symptomatic hyperviscosity syndrome treated with pentostatin. This patient responded partially to plasmapheresis, but improved rapidly on pentostatin at a dose of 4 mg/m² given at weekly intervals. This response lasted for only one month following discontinuation of therapy.

**Purine Nucleoside Analogs in Combination with Other Agents**

Despite the confirmed activity of the purine analogs against lymphoid malignancies, the majority of patients treated with these agents as initial or salvage therapy are destined to relapse. Because the purine analogs interfere with DNA repair, their addition to traditional DNA damaging chemotherapy might augment the tumoricidal effect of the drugs. Clinical activity and toxicities of such combinations are summarized in Table 5.

These studies demonstrate that the purine analogs can be incorporated safely into regimens including traditional chemotherapy with acceptable toxicity [104-112]. The major toxicities are myelosuppression and infection, which can become severe in heavily pretreated patients. The maximum tolerated dose of fludarabine when combined with other agents is usually 75 mg/m² per cycle, although doses up to 120 mg/m² have been tolerated without significant morbidity [107]. 2-CdA has been administered safely in combination regimens at doses of 4 mg/m²/day for three to seven days.

**Cross-Resistance Among Purine Nucleoside Analogs**

Saven et al. [113] reported on five patients with HCL who were refractory or intolerant to pentostatin and responded to treatment with 2-CdA. Although no other study has focused on the issue of cross-resistance among nucleoside analogs in HCL, the available reports show that 13 out of 17 patients failing pentostatin responded to salvage therapy with 2-CdA [36, 43-45, 113] (Table 6).

<table>
<thead>
<tr>
<th>Author</th>
<th>Medication</th>
<th>Schedule</th>
<th>Patients (#)</th>
<th>Untreated (#)</th>
<th>RR (CR) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delannoy [101]</td>
<td>2-CdA</td>
<td>4 mg/m²/d CIV × 7 d (5 Pts)</td>
<td>18</td>
<td>5</td>
<td>39 (0)</td>
</tr>
<tr>
<td>Dimopoulos [102]</td>
<td>2-CdA</td>
<td>0.1 mg/kg/d CIV × 7 d × 2</td>
<td>26</td>
<td>26</td>
<td>85 (12)</td>
</tr>
<tr>
<td>Dimopoulos [100]</td>
<td>2-CdA</td>
<td>0.1 mg/kg/d CIV × 7 d × 2</td>
<td>29</td>
<td>9</td>
<td>59 (4)</td>
</tr>
<tr>
<td>Dimopoulos [99]</td>
<td>FLD</td>
<td>20-30 mg/m²/d × 3-5 d q4w</td>
<td>28</td>
<td>2</td>
<td>36 (4)</td>
</tr>
<tr>
<td>Bruera [103]</td>
<td>DCF</td>
<td>4 mg/m²/week × 5</td>
<td>1</td>
<td>0</td>
<td>100 (0)</td>
</tr>
</tbody>
</table>

RR: Response rate; CR: Complete Response; CIV: Continuous i.v. infusion; FLD: Fludarabine; DCF: Pentostatin; Pts: Patients.

*Range of courses administered 1-6 (median 2).

*Range of courses administered 1-24 (median 6).
In contrast to the results in HCL, only 10 of 76 CLL patients treated with 2-CdA after fludarabine failure responded [67, 71, 92, 114-118] (Table 6). Overall, there appears to be significant cross-resistance among these agents in CLL, and a second purine analog should not be used routinely for the treatment of relapsed disease after the failure of another nucleoside analog.

### TOXICITIES OF THE PURINE NUCLEOSIDE ANALOGS

Significant side effects, including severe neurotoxicity, were observed in the initial phase I studies using high doses of the purine analogs [119]. At the currently recommended doses of these agents, the most commonly seen toxicity is myelosuppression. Although the evaluation of hematologic toxicity can be difficult in patients with lymphoproliferative disorders due to bone marrow involvement by tumor, it appears that purine analogs produce transient decreases in both neutrophils and platelets in 30%-80% of patients [68, 69, 71, 72, 75, 76, 78-80, 83, 84, 96, 98, 120]. At least 50% of CLL patients treated with fludarabine have an absolute neutrophil count less than 1,000/µl [54]. Significant thrombocytopenia, usually defined as a platelet count less than 50-100,000/µl, is seen in 17%-26% of fludarabine-treated patients [58, 121]. The rate of thrombocytopenia ranges from 14%-40%. In the largest single study with pentostatin, Mercieca et al. [79] found a 20% incidence of grade 3-4 neutropenia and a 25% incidence of grade 3-4 thrombocytopenia. The degree of myelosuppression induced by the purine analogs correlates with the extent of previous treatment, the initial disease stage and the response to treatment.

In contrast to the results in HCL, only 10 of 76 CLL patients treated with 2-CdA after fludarabine failure responded [67, 71, 92, 114-118] (Table 6). Overall, there appears to be significant cross-resistance among these agents in CLL, and a second purine analog should not be used routinely for the treatment of relapsed disease after the failure of another nucleoside analog.

**Table 5. Purine nucleoside analogs in combination with other agents**

<table>
<thead>
<tr>
<th>Author</th>
<th>Schedulea</th>
<th>Disease</th>
<th>Patients (#)</th>
<th>RR (CR) %</th>
<th>Toxicities (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weiss [104]</td>
<td>Chl 20 q2w &amp; F 10 × 5 d</td>
<td>CLL</td>
<td>15</td>
<td>27 (7)</td>
<td>Chl q2w: 2/6 patients died Chl q4w: 1/9 patients died (F 20 × 5)</td>
</tr>
<tr>
<td>Foss [105]</td>
<td>DCF 4 mg/m²/d × 3 d &amp; IFN 10 MU/m² IM d22 and 50 MU/m² d23-26 q4 d</td>
<td>CTCL</td>
<td>41</td>
<td>41 (5)</td>
<td>N/V 12, Fever 10, Fatigue 20, ANC 36, Creatinine 2.5, MS A 17</td>
</tr>
<tr>
<td>Foss [106]</td>
<td>F 25 × 5 d &amp; IFN 5 MU/m² SC tiw</td>
<td>CTCL</td>
<td>35</td>
<td>51 (11)</td>
<td>Fever 9, Fatigue 12, Infection 20, ANC 88, PLT 17, Hgb 17, Neurologic B 3</td>
</tr>
<tr>
<td>Robertson [107]</td>
<td>F 25-30-3 4-4 d, DOX 50 mg/m²</td>
<td>CLL</td>
<td>29</td>
<td>59 (17)</td>
<td>Infections 38, FUO 10, Deaths 7, Decrease in EF 7</td>
</tr>
<tr>
<td>Elias [108]</td>
<td>I: Chl 20, F 10 × 5 d</td>
<td>CLL</td>
<td>21</td>
<td>53</td>
<td>I: Hgb 13, PLT 25, Cardiac 13</td>
</tr>
<tr>
<td>McLaughlin [109]</td>
<td>F 20-25 × 2-3 d, N 8-12 mg/m² d1, D 20 mg/d × 5 d</td>
<td>NHL</td>
<td>21</td>
<td>71 (43)</td>
<td>Median nadir ANC 0.7-2.0/mm³</td>
</tr>
<tr>
<td>Tefferi [110]</td>
<td>2-CdA 1-4 mg/m²/d CIV × 7 d q4w &amp; Chl 30 q2w</td>
<td>CLL/NHL</td>
<td>15</td>
<td>20 (7)</td>
<td>Moderate-severe ANC 53, PLT 67 F &amp; N 33</td>
</tr>
<tr>
<td>Zullian [111]</td>
<td>2-CdA 0.1 mg/kg/d SC × 3-5 d, CPA 500 mg/m² d1, PRD 40 mg/m²/d × 5 d</td>
<td>CLL/NHL</td>
<td>7</td>
<td>86 (72)</td>
<td>No acute side effects</td>
</tr>
<tr>
<td>Tobinai [112]</td>
<td>DCF 5 mg/m²/w × 3 &amp; VAEP</td>
<td>ATLL</td>
<td>61</td>
<td>48 (30)</td>
<td>No grade 4 ANC or PLT toxicity</td>
</tr>
</tbody>
</table>

**Legend:**
- RR: Response rate; CR: Complete response; Chl: Chlorambucil; F: Fludarabine; DCF: Pentostatin; IFN: Interferon; DOX: Doxorubicin; PRD: Prednisone; N: Mitoxantrone; D: Dexamethasone; CPA: Cyclophosphamide; CTCL: Cutaneous T cell lymphoma; CLL: Chronic lymphocytic leukemia; NHL: Non-Hodgkin's lymphoma; ATLL: Adult T cell leukemia/lymphoma; IM: Intramuscular; SC: Subcutaneous; CIV: Continuous intravenous infusion; VAEP: Vincristine 1 mg/m² d1,8, Doxorubicin 40 mg/m² d1, Etoposide 100 mg/m² d1-3, Prednisolone 40 mg/m² d1-2; N/V: Nausea/vomiting; ANC: Absolute neutrophil count; PLT: Platelets; Hgb: Hemoglobin; MS: Mental status changes; EF: Ejection fraction; F&N: Fever and neutropenia; NS: Not stated.
- Fludarabine doses are expressed in mg/m²/d, and chlorambucil doses in mg/m².
- Ten patients also received prednisone 30 mg/m².
- All toxicities listed are grades 3-4.
- All toxicities listed are grades 3-5.
- Asymptomatic.

By guest on October 18, 2017
In patients with HCL treated with 2-CdA, nonhematologic toxicity is usually limited to febrile episodes. Some of these episodes, which coincide with the rapid decline in the number of hairy cells and last for two to ten days, are thought to be related to the release of cytokines from lysed tumor cells [122]. Serious bacterial infections, such as pneumonia or sepsis, are observed in 3%-6% of patients, and usually occur early in the treatment course [36, 42, 43, 48]. The risk of infection following purine analog use increases with myelosuppression, but severe infectious complications can occur in the absence of neutropenia [121]. Serious bacterial infections, such as pneumonia or sepsis, are observed in 3%-6% of patients, and usually occur early in the treatment course [36, 42, 43, 45, 48].

The risk of infection following purine analog use increases with myelosuppression, but severe infectious complications can occur in the absence of neutropenia [121]. The risk of infection also increases with extent of prior treatment, especially therapy with other nucleoside analogs and steroids [59, 72, 92, 106, 114, 118]. However, the infection rates during and following purine analog treatment and during and following traditional chemotherapy have not been compared in a randomized trial. The recently completed intergroup trial of fludarabine versus chlorambucil in patients with CLL will address this question directly. Moreover, in the long-term follow-up of CLL patients treated with fludarabine as first-line therapy at the MD Anderson Cancer Center (Houston, TX) [64], only one febrile episode was noted per 3.5 patient years at risk among patients in remission.

Another concern regarding the purine analogs stems from their ability to induce substantial and prolonged suppression of the helper (CD4) T cell population. After treatment with fludarabine for CLL, O’Brien et al. [59] found that the CD4 count decreased from a median of 1,015/µl at baseline to 169/µl in three months and 148/µl in six months. Dimopoulos et al. [102] showed that in nine patients with WM who responded to 2-CdA and remained in remission, CD4 counts remained low at 150/µl one year after treatment. Similar results have been reported with pentostatin in patients with HCL [123].

In phase I studies, severe and even fatal neurologic syndromes were associated with the use of the purine analogs [119]. In contrast, neurotoxicity is seen in only 15% of patients treated with purine analogs at standard doses. Moreover, the symptoms are usually mild and reversible, such as confusion during infusion or paresthesias. Severe or life-threatening toxicity is seen in approximately 1% of patients treated with any of the purine analogs [124]. Unfortunately, there are no specific pretreatment characteristics stratifying patients into different risk categories for subsequent neurotoxicity.

Nonhematologic toxicity other than infection and neurotoxicity is uncommon. Nausea, vomiting, alopecia and fatigue occur in approximately 5% of cases treated with fludarabine or 2-CdA and are almost never severe in nature. Tumor lysis, although rare, has been reported with these agents in the treatment of CLL [52, 60, 125-127]. Pentostatin is associated with a higher incidence of nonhematologic toxicity, which is dose dependent. With the currently used doses, grade 3-4 gastrointestinal side effects are rare [33, 36, 37]. Other toxicities which can be encountered with the low dose schedules include transient elevations of serum creatinine (0.6%-13%) [33, 37, 74], liver function test abnormalities (0.6%-9%) [33, 37, 74], mild conjunctivitis (21%-26%) [33, 74] and skin rash (4.5%-35%) [33, 36].

**CONCLUSIONS**

The availability of the purine analogs fludarabine, 2-CdA (cladribine) and pentostatin represents significant progress in the treatment of low-grade lymphoid malignancies. Fludarabine is approved by the FDA for the therapy of alkylator-relapsed CLL, pentostatin is approved for interferon-failed HCL, and 2-CdA is approved for untreated or failed HCL. However, their exact role in the treatment of low-grade lymphoproliferative disorders other than HCL remains to be defined.

The addition of purine analogs to regimens employing standard chemotherapy has the potential to provide synergistic cytotoxicity. The purine analogs demonstrate moderate myelosuppression, allowing for combinations with traditional myelotoxic agents. Moreover, by interfering with DNA repair mechanisms, the nucleoside analogs may
complement the DNA-damaging effects of standard chemotherapy. Such combinations may be very toxic, and carefully designed phase I studies need to define the exact doses to be used.

In many cases, despite a histologic CR, residual disease can be detected with sensitive techniques. Therefore, strategies aiming to improve the long-term results of patients with low-grade lymphoproliferative disorders should attempt to “consolidate” the initial response obtained with nucleoside analogs. Unfortunately, a recent study from the MD Anderson Cancer Center [128] showed that in 22 CLL patients in partial or complete remission after fludarabine, treatment with IFN-α at a dose of 3 × 10^6 units three times weekly did not eradicate residual disease in the complete responders, converted only one out of the nine partial responders to a complete responder and did not increase the time to progression compared to historical controls. Other therapies, such as interleukin 2 or immunotoxin therapy, directed against minimal residual disease could be employed as well. In a trial at the MD Anderson Cancer Center, the immunotoxin anti-B4-blocked ricin (anti-B4-bR) has been used following fludarabine therapy for patients with CLL in an attempt to eradicate minimal residual disease. Alternatively, patients with good quality initial responses may be treated on high-dose chemoradiation protocols in an effort to further cytoreduce the residual tumor burden. The long-term results of such aggressive therapies are lacking at the present time.

More than simply increasing RRIs, the purine analogs have raised the enthusiasm of the medical community for exploring new treatments for the low-grade lymphoid malignancies. Significant tumor reductions can be obtained with tolerable toxicity on an entirely outpatient basis. Future investigations in this area should address the issue of immunodeficiency, establish safe combination regimens with increased activity and also identify postremission strategies that would prolong the long-term survival of these patients or even lead to cure.

ACKNOWLEDGMENT

Michael L. Grossbard is the recipient of a clinical research grant from Berlex, Inc.

REFERENCES


79 Mercieca J, Matutes E, Dearden C et al. The role of pentostatin in the treatment of T-cell malignancies: analysis of...


