Combination Therapy With Purine Nucleoside Analogs

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Pentostatin (Nipent) has demonstrated significant activity as a single agent in patients with low-grade B- and T-cell lymphomas, but thus far, clinical experience with combinations of pentostatin and other agents is limited. A study of alternating administration of pentostatin and high-dose interferon-alfa-2a (Roferon A) in cutaneous T-cell lymphoma patients has been undertaken and has demonstrated a 41% response rate, with tolerable toxicity. Studies combining pentostatin with alkylating agents, including chlorambucil (Leukeran) and cyclophosphamide (Cytoxan, Neosar) in patients with chronic lymphocytic leukemia (CLL) have reported significant immunosuppression and have required dose modifications of one or both agents. Recently, a clinical trial was initiated to evaluate the combination of pentostatin and cordycepin, a novel purine analog, in patients with terminal deoxynucleotidyl transferase–positive acute lymphocytic leukemia, based on in vitro data demonstrating the significant synergy of this combination. [ONCOLOGY 14(Suppl 2):31-35, 2000]

Pentostatin (Nipent) has shown single-agent activity in patients with low-grade T-cell and B-cell non-Hodgkin’s lymphomas.[1-3] High durable response rates have been achieved in patients with cutaneous T-cell lymphoma. [4-7] Studies have shown a trend toward improved response rates in patients who were previously untreated or who had received only topical therapies, although most of the patients entered into these trials had refractory disease. The toxicities were comparable among the studies, with greater hematologic toxicity seen in studies that used more intensive dosing regimens.

Pentostatin in Combination With Alkylating Agents

Thus far, the experience combining pentostatin (Nipent) with other cytotoxic chemotherapeutic agents has been limited. A recent Eastern Cooperative Oncology Group (ECOG) study evaluated the combination of pentostatin (2 to 4 mg/m² on day 1) with chlorambucil (Leukeran 30 mg/m² on day 1) and prednisone (80 mg on days 1 to 5 of a 14-day cycle) in patients with B-cell chronic lymphocytic leukemia (B-CLL) who were in sensitive first relapse or were previously untreated.[8] Because of the increasing toxicity encountered at higher doses, 2 mg/m² was selected as the phase II dose, and 43 patients were treated at this dose level.

The overall response rate was 87%, with a median response duration of 32 months. Grade 3 or 4 infections occurred in 33% of patients, and included *Pneumocystis* pneumonia in one patient and fungal pneumonia in two. Herpes zoster developed in 10 patients. Although this combination was active, the incidence of opportunistic infections was felt to be unacceptable. A subsequent trial incorporated antibacterial and antiviral prophylaxis, while omitting prednisone to reduce the infection rate.[9]

Dose-Escalation Trial

A dose-escalation trial examined the use of pentostatin and cyclophosphamide (Cytoxan, Neosar) in patients with high-risk CLL who had failed to respond to other therapies, including fludarabine (Fludara). [M. Weiss, personal communication, November 1999] The pentostatin dose remained fixed
at 4 mg/m² and the cyclophosphamide dose was escalated, with the first cohort receiving 600 mg/m². Thus far, seven patients have been treated, three at 600 mg/m², and four at 900 mg/m² of cyclophosphamide. The cycles are repeated every 21 days.

All patients had biochemical evidence of tumor lysis following the first cycle of therapy. Of three patients treated at the first dose level, two achieved partial responses (PRs) and one a complete response (CR). All of these responders had previous exposure to fludarabine- and alkylating agent-containing regimens. One patient, who was treated at the 900 mg/m² dose level, died of progressive disease. Overall, the regimen was well tolerated, and adverse events included mild fatigue, nausea, and vomiting. Further study of this combination at higher dose levels is underway.

**Pentostatin and Interferon**

Combination therapy with pentostatin and interferon (IFN)-alfa was first used in the setting of hairy cell leukemia to determine whether there would be an improvement in response rates by combining two active agents. The regimen was well tolerated. Based on the high response rates to treatment with pentostatin as a single agent in cutaneous T-cell lymphoma patients, a similar study was initiated to explore the combination of pentostatin and intermittent high-dose IFN-alfa-2a (Roferon A).

This study enrolled 41 refractory cutaneous T-cell lymphoma patients with advanced skin or visceral disease. The median age was 59 years. Cutaneous tumors were present in 15 patients, and 13 had erythroderma. Visceral involvement was noted in seven patients, and 24 presented with blood involvement, defined as greater than 20% of lymphocytes appearing atypical with convoluted nuclear contours on peripheral smear. Most patients had failed to respond to multiple previous therapies, with 25 having failed both chemotherapy and total-skin electron-beam irradiation. Topical therapies only had been applied to six patients, and six had received no prior therapy.

The dose and schedule of pentostatin used in this study, 4 mg/m² daily for 3 consecutive days, was based on reports from prior phase II studies that demonstrated the efficacy and tolerability of this dose level. Interferon-alfa-2a was administered intramuscularly at a dose of $10 \times 10^6$ U on day 22 and $50 \times 10^6$ U on days 23 to 26. An alternating schedule of pentostatin and IFN-alfa had previously proven to be well tolerated in patients with hairy cell leukemia. This 42-day cycle was repeated for up to 12 months, or until disease progression or intolerable toxicity occurred. However, the design of the study allowed patients who were intolerant of one of the drugs to continue to receive the other drug on an every-21-day schedule.

**Response Rates**

The overall response rate was 41%, with two CRs and 15 PRs. Both patients who achieved a CR had Sézary syndrome and diffuse erythroderma prior to treatment. With treatment, all skin lesions and circulating cells disappeared. A third CR, diagnosed at autopsy in a patient with extensive plaque disease, was initially recorded as a PR based on persistent skin abnormalities. The patient died of gastrointestinal hemorrhage 3 months after completing therapy. Another CR occurred in a patient thought to have stable disease after four cycles of therapy; this patient refused further treatment and was found on routine follow-up 2 months later to have no evidence of disease.

Responses were noted in all sites of disease, except visceral sites. There was no correlation between response and skin stage (T1,2 vs T3,4), presence of blood involvement, or lymph-node stage (LN2,3 vs LN4), although patients with erythroderma had a higher response rate (8 of 18) than those of any other skin stage (similar to studies with single-agent pentostatin). The correlation between prior therapy and response was not statistically significant ($P = .045$), unlike earlier studies with pentostatin, which suggested a higher response rate in untreated patients.

The overall survival duration was 15.8 months, with a trend toward improved survival in patients who had had no prior therapy (29 months) vs those who had received prior therapy (15 months). As
listed in Table 1, the median progression-free survival duration was 13.1 months, which compares favorably with that of single-agent IFN studies[12] and combination regimen studies,[13] and is superior to the survival duration reported in our study of IFN and fludarabine.[14]

Toxicity Profile

The combination of pentostatin and IFN-alfa was well tolerated, as demonstrated by the fact that the median projected dose of pentostatin delivered was 92.8%, with a mean of five courses per patient (range, 1 to 12), and the median projected dose of IFN-alfa was 81%, with a mean of three cycles delivered (range, 1 to 8). Pentostatin was discontinued in two patients due to toxicity, but was not discontinued in any patients due to disease progression. Interferon was discontinued in eight patients due to toxicity, although these patients continued therapy with pentostatin alone.

The most frequently observed grade 3-4 toxicity was hematologic, with granulocytopenia occurring in 15 of 41 patients. Although pentostatin therapy has been shown to be immunosuppressive, only eight patients developed opportunistic infections. Disseminated herpes zoster developed in seven patients, one had central nervous system toxoplasmosis and cytomegaloviral pneumonia, and six acquired bacterial sepsis. Nausea occurred in five patients, and reversible central nervous system events including confusion and headache in seven.

After infusion with pentostatin, three patients experienced reversible bronchospasm. One of these patients had persistent restrictive and obstructive defects on pulmonary function tests 1 year later. Another developed reversible bronchospasm and pulmonary edema 6 days after completion of the third cycle of pentostatin. Preclinical animal toxicity studies demonstrated pentostatin-associated pulmonary hypersensitivity pneumonitis and nodular pulmonary fibrosis, but other clinical trials to date have not demonstrated significant pulmonary toxicity even at higher doses. It is unclear what role the use of IFN in this study played in predisposing patients to pulmonary toxicity.

In summary, this study demonstrated that the combination of pentostatin and intermittent high-dose IFN was well tolerated and was associated with a high response rate and an impressive response duration, especially in patients with Sézary syndrome. The frequent incidence of granulocytopenia may have been due to the high-dose of IFN, as most patients who discontinued therapy due to toxicity stopped taking IFN, not pentostatin.

Fludarabine and Interferon

In a subsequent study, we treated patients with advanced or refractory cutaneous T-cell lymphoma with the combination of fludarabine phosphate and low-dose IFN.[14] Of the 35 evaluable patients, 21 had not responded to prior chemotherapy or total-skin electron-beam irradiation, seven had received prior topical therapy, and seven were untreated. Advanced skin disease (tumors or erythroderma) was present in 31 patients, and 19 had circulating tumor cells in the peripheral blood. Ten patients had received prior pentostatin therapy.

Fludarabine was administered at a dose of 25 mg/m² daily for 5 consecutive days, and IFN at a dose of 5 x 10⁶ U/m² 3 times per week with a dose escalation to 7.5 x 10⁶ U/m² on day 29 if no grade 3 toxicity was noted. The overall response rate was 51%, with four CRs and 14 PRs. Among patients who had received prior pentostatin, five (50%) achieved a PR. Univariate analysis demonstrated no statistically significant association between response and skin stage, presence of blood involvement, or prior therapy, similar to the results of the pentostatin-IFN study.

Yet, while response rates were similar in the two studies, the response duration with the fludarabine-IFN combination was significantly shorter (median time to progression was 5.9 months). The time to progression for the responders who had received prior pentostatin ranged from 3 to 6 months. The response durations for the combination of fludarabine and IFN are similar to those of single-agent fludarabine in phase II studies (range, 2 to 17 months).[15]

The incidence of hematologic toxicity was significantly higher with the fludarabine-IFN combination than with the pentostatin-IFN combination, perhaps because of the different spectrum of activity of
fludarabine compared with pentostatin. The incidence of grade 3 or 4 neutropenia was 60%, with grade 4 anemia and thrombocytopenia reported in 11%, as compared with 36% in the pentostatin-IFN study. Prolonged nadirs occurred early in this trial, compared to trials using fludarabine alone; treatment delays were noted after a median of five cycles of fludarabine.[16] One patient developed bone marrow aplasia and remained transfusion-dependent until death 4 months later. Septicemia developed in six patients and opportunistic infections in five, similar to the pentostatin-IFN study.

**Different Spectra of Activity**

These results suggest different spectra of activity and toxicity when pentostatin or fludarabine are combined with IFN. The higher incidence of hematologic toxicity in the fludarabine-IFN study suggests that the IFN may have unfavorable effects on bone marrow hematologic recovery after purine analog administration. However, this occurs to a less significant degree with pentostatin than with fludarabine. The high incidence of infections in both studies may be attributable not only to myelosuppression but also to the underlying immunologic deficits in patients with cutaneous T-cell lymphoma. Anergy studies indicated that over 80% of the patients entered in the fludarabine-IFN study were anergic at study entry.

While overall time to progression was significantly different in the two studies, it is notable that response durations of 37+ months for complete responders were reported in both studies. The overall differences in response durations cannot be attributed to variations in the patient populations, as shown in Table 2, but rather may reflect the differences in biological activities among the purine analogs. It might be worthwhile to further investigate the combination of pentostatin with either sequential or simultaneous low-dose IFN earlier in the disease course of cutaneous T-cell lymphoma patients. Skin testing for anergy might be a predictor of infectious complications, and future studies should include prophylaxis against herpes and *Pneumocystis carinii* infections.

**Pentostatin and Cordycepin**

Early combination studies of pentostatin and other purine analogs, including fludarabine, were associated with significant toxicity in the form of bone marrow suppression and neurotoxicity.[M. Grever, personal communication, October 1999] We recently initiated a combination study of pentostatin and a novel nucleoside analog, cordycepin (3'-deoxyadenosine [3'-dA]) in patients with refractory terminal deoxynucleotidyl transferase (TdT)-positive leukemia.[15] Prior studies demonstrated that cordycepin, when protected from adenosine deaminase (ADA) by coformycin or pentostatin, is specifically cytotoxic to leukemia and lymphoma cells that express the enzyme TdT. Terminal deoxynucleotidyl transferase is a DNA polymerase that will catalyze the addition of any deoxyribonucleotide to any 3'-OH end of a preformed oligo- or polydeoxynucleotide initiator, in a template-independent manner.[16]

The expression of TdT is restricted to subsets of primitive lymphocytes in the bone marrow and thymus pre-B and pre-T cells. In disease states, TdT is expressed in the blast cells of acute lymphoblastic leukemia, lymphoblastic lymphoma, and in lymphoblastic blast crisis of chronic myelogenous leukemia.[17-19]

In vitro studies demonstrated that cordycepin, in concentrations as low as 1 mmol/L and in the presence of pentostatin, was toxic to all TdT-expressing cell lines, and these results were reproduced in vivo in severe combined immunodeficiency (SCID) mice bearing a TdT-positive human xenograft. The mechanism by which cordycepin is selectively toxic only to TdT-positive cells has not yet been fully elucidated, but preliminary data suggest that the TdT-positive cells are undergoing apoptosis.

**Inhibiting ADA Activity**

In this clinical trial, pentostatin is being used to inhibit ADA and thus prevent the degradation of the cordycepin. The initial phase of this protocol was designed to determine the minimal concentration of pentostatin necessary to inhibit 75% of the detectable ADA activity in plasma, red blood cells, and
lymphocytes from treated patients. In prior studies, a single bolus injection of 3 mg/m² of pentostatin resulted in 90% inhibition of ADA in plasma, red blood cells, and buffy coat cells at 15 minutes, with a plasma enzyme recovery half-life of 8.5 hours and full recovery by 24 hours.[20-22]

To determine the lowest pentostatin dose that could produce this degree of ADA inhibition, the effects of lower doses of pentostatin (2 and 3 mg/m²) on ADA activity were explored in the first two cohorts of the study.[23,24] Adenosine deaminase levels were determined in plasma, red blood cells, and buffy coat leukocytes by descending paper chromatography, with the level of ADA activity (the conversion of adenosine to inosine) calculated from the ratio of inosine to adenosine plus inosine.

In the first dose group, three patients were treated with pentostatin (2 mg/m²) and cordycepin (6 mg/m²) daily for 3 days. At this dose of pentostatin, sufficient ADA inhibition was not observed in any of the treated patients, as shown in Table 3. In the second dose group, three patients were treated with pentostatin (3 mg/m²/d) and cordycepin (6 mg/m²/d). Adenosine deaminase was inhibited by 90% in all patients, and there were transient decreases in circulating blasts. Of the seven patients treated with pentostatin (3 mg/m²), all demonstrated ADA inhibition. Cordycepin doses have been escalated to 24 mg/m², with no significant toxicity observed thus far.

A 43-year-old male patient (patient number 10) with acute lymphocytic leukemia demonstrated a significant decrease in his circulating blast count, from 77,000 to 2,100 cells/µL, as well as a restoration of normal hematopoiesis after receiving 3 days of therapy with cordycepin (24 mg/m²) and pentostatin (3 mg/m²). This patient has continued to demonstrate a clinical response to subsequent cycles of therapy.

Thus far, only mild toxicities have been observed. Several patients experienced grade 2 nausea, and fluctuations in blood glucose and calcium have been observed. There has been no hematologic or neurologic toxicity, as was noted in prior studies combining other purine analogs. This study is ongoing.

**Summary and Future Directions**

The purine analogs, including pentostatin, fludarabine, and 2´-chlorodeoxyadenosine (cladribine [Leustatin]), have shown promise as single agents and in combination in lymphoid hematologic malignancies. Combinations of purine analogs with alkylating agents, such as cyclophosphamide or chlorambucil, have been associated with higher response rates, but the increasing incidence of infectious complications and opportunistic infections has demanded dose adjustments. Because purine analogs have been shown to interfere with DNA repair and may thus inhibit repair of damage induced by alkylating agents, further investigation of the potential in vivo synergy using combinations of these drugs is of interest.

The experience with the combination of purine analogs and biological agents has been limited thus far to studies with IFN-alfa. Intermittent IFN schedules have been tolerated better than continuous dosing schedules due to cumulative myelosuppression. While doses up to pentostatin 5 mg/m2 for 3 consecutive days have been well tolerated in single-agent studies, lower-dose regimens should be explored in future combination studies.

In vitro studies are currently underway to investigate the potential synergy between pentostatin and other T-cell–targeted therapeutic agents, including immunotoxins.

**References:**


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