Recombinant Beta-Serine-Interferon in Hairy Cell Leukemia Compared Prospectively With Results With Recombinant Alpha-Interferon

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Ten patients with hairy cell leukemia (HCL) requiring therapy were treated with recombinant beta-serine-interferon (rIFN-Bser) (90 × 10^6 units [U] subcutaneously three times a week). Eight patients were evaluable for response and nine for toxicity. Five patients (63%) showed normalization of peripheral blood counts, and an additional two patients (25%) showed improvement in at least one hematologic variable. Persistent hairy cells were detected in the bone marrow of all patients at the completion of therapy. All patients experienced influenza-like symptoms which were not dose limiting and which resolved with continued therapy. Erythema and induration at interferon injection sites developed in five patients (56%); one required dose reduction and another was removed from the study for this reason. Data from matched historical controls treated with recombinant alpha-interferon are presented for comparison. We conclude that rIFN-Bser has activity in HCL.

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Hairy cell leukemia (HCL) is a lymphoproliferative disorder characterized by pancytopenia, splenomegaly, circulating lymphocytes containing the tartrate-resistant isoenzyme of acid phosphatase (TRAP), and infiltrated bone marrow that is difficult to aspirate. Until recently, splenectomy was the only effective treatment for HCL patients requiring therapy. Currently, alpha-interferons and pentostatin have shown efficacy in improving hematologic variables in patients with HCL.

Alpha-interferons and beta-interferons are type I interferons, sharing approximately 40% amino acid homology and acting on cells through a common receptor. In vitro, both alpha-interferons and beta-interferons inhibit proliferation by hairy cells stimulated by B-cell growth factor. We report the results of a Phase II trial of recombinant beta-serine-interferon (rIFN-Bser) in patients with HCL requiring interferon therapy.

Due to emerging data at the time this trial was initiated suggesting that alpha-interferon represented the treatment of choice for HCL requiring therapy and failing to respond to splenectomy, each patient entered on the rIFN-Bser trial was paired at the time of entry with a patient from the recombinant alpha-2b-interferon (rIFNα2b) study we reported previously. Patients were matched as closely as possible for hematologic variables, splenectomy status, sex, age, and infection history. The matching was done prospectively, before the initiation of beta-interferon therapy, and “blindly,” without knowledge of the ultimate treatment outcome for the chosen alpha-interferon patients. Following these cohorts closely as treatment progressed allowed us to continually assess the relative efficacy of the two preparations with regard to the ethical basis for continuing the trial of rIFN-Bser. Data from this selected recombinant alpha-interferon cohort also are presented.

Materials and Methods

Criteria for inclusion included the following: (1) pathologically confirmed HCL; (2) an indication for treatment, including significant cytopenia (hemoglobin < 10 g/dl, platelet count < 100,000 × 10^9/l, or absolute neutrophil count < 1000 × 10^3/l), or absolute neutrophil count < 1000 × 10^3/l), transfusion dependence, or opportunistic infection; (3) failure or refusal of splenectomy; (4) no therapy with androgens, corticosteroids, or cytotoxic drugs for 1 month before entry; (5) acceptable renal and hepatic function; (6) no serious cardiac disease; (7)
no synchronous malignancy; and (8) no prior interferon therapy. Signed informed consent approved by the UCLA Human Subjects Protection Committee was obtained from all patients. All patients presenting to UCLA with HCL during the period that the trial was open to accrual (November 1985 to April 1987) met inclusion criteria and were entered on study; all patients treated are reported on here. No selection or randomization took place and no patients were lost to follow-up. Ten patients began rIFN-βser therapy, and this met the target accrual.

Patients were treated with rIFN-βser (Betaseron, Triton Biosciences, Emeryville, CA) at a dose of 90 x 10^6 units (U) three times a week. The first three patients were started on intravenous therapy (90 x 10^6 U daily for 14 days every 4 weeks). To improve patient convenience, the protocol was amended and changed to three times a week by subcutaneous route. Other patients were treated with 90 x 10^6 U three times a week subcutaneously from the initiation of therapy. This dose was chosen based on Phase I and II clinical trials in other diseases that documented that it was associated with acceptable toxicity. The first week of injections was done under our supervision. After being taught how to administer the drug themselves, all subsequent doses were self-administered by the patients at home.

In our previous alpha-interferon series, similar entry criteria applied. These patients were treated with an rIFNα2b (Intron-A, Schering Corporation, Kenilworth, NJ) dose of 2 x 10^6 U/m^2 subcutaneously three times a week. In both series, therapy was continued for 12 months in responding patients unless high-grade toxicity or disease progression mandated earlier discontinuation. Therapy was continued for at least 6 months before patients were designated as nonresponders. Patients were premedicated before each dose of interferon with 975 mg of acetaminophen. Patients were seen monthly at UCLA by one of the authors (J.G.). Complete blood counts, differentials, urinalysis, and liver function tests were checked weekly for the first month of therapy and monthly after that. Unilateral bone marrow biopsy specimens and TRAP stains were obtained every 3 months. Patients kept written records of symptoms and medication compliance. Strict drug accountability was practiced.

Response criteria used in data analysis were as follows: (1) a complete response (CR) required regression of all organomegaly, absence of hairy cells from peripheral blood and bone marrow biopsy specimens, and restoration of hematologic variables (hemoglobin > 12 g/dl, platelets > 100 x 10^9/l, and absolute neutrophil count > 1.5 x 10^9/l); (2) a pathologic partial response (PPR) required reduction of organomegaly by at least 50%, reduction in circulating hairy cells to less than 5% of leukocytes present, regression of hairy cells in bone marrow by at least 50%, and restoration of hematologic variables as defined above; (3) an hematologic partial response (HPR) was defined as restoration of all hematologic variables to levels as defined above, with less than a 50% reduction in bone marrow hairy cells; and (4) a minor response (MR) was restoration of at least one previously abnormal hematologic variable to normal.

Serum samples for the analysis of neutralizing activity against beta-interferon were taken from all patients before starting therapy, monthly during therapy, and once more between the 23rd and 24th days after the last interferon dose. Samples were screened in triplicate with an enzyme-linked immunosorbent assay (ELISA) for immunoglobulin (Ig) G and M antibodies binding rIFN-βser. Any samples positive by ELISA for binding antibody were to be screened in triplicate for rIFN-βser-neutralizing antibody activity in an antiviral assay.

**Results**

Ten HCL patients were treated with beta-interferon; one died of an infection within 2 weeks of beginning therapy and was not evaluable (no toxicities were observed in this patient). Another patient was taken off of the drug after 8 weeks because of large, nonhealing ulcers at the injection site (this patient was evaluable for toxicity but not response). Clinical and prognostic characteristics before therapy for the remaining eight patients and their matched cohorts from the alpha-interferon series are shown in Table 1. The beta-interferon and alpha-interferon groups were well matched for age, sex, and history of opportunistic infections.

None of the beta-interferon patients achieved a CR. Two (25%) patients achieved a PPR and three (38%) achieved an HPR. Two patients (25%) achieved an MR (one because of persistent mild anemia after the completion of therapy). Hence, 63% of our patients demonstrated a clear objective improvement in all cell lines and an additional 25% showed some benefit from beta-interferon therapy. One patient who achieved an HPR after 5 months of beta-interferon therapy showed progression of disease with continued therapy and subsequently lost that response. Serum from this patient did not have neutralizing activity against rIFN-βser in viral-induced plaque inhibition assays. This patient has subsequently shown a CR with pentostatin therapy.
By comparison, in the alpha-interferon group, there was one (13%) CR, two (25%) PPR, three (38%) HPR, and two (25%) MR. In these matched patients treated with alpha-interferon, 75% showed a clear objective improvement in all cell lines and the remaining 25% showed some benefit from therapy. By chi-square analysis, there was no statistically significant difference between the alpha-interferon and beta-interferon groups in terms of overall response rate.

The mean hematologic variables for the two groups are shown in Table 2 (these are shown graphically in Figure 1), together with changes in bone marrow cellularity, percent hairy cells, and hairy cell index (total cellularity \times percent hairy cells). Means include only those patients remaining on therapy after the given duration of therapy. In the alpha-interferon group, all eight patients completed 12 months of therapy. In the beta-interferon group, one patient (HPR) died of pneumonia after 4 months of therapy, one (NR) was taken off of therapy after 7 months because of failure to respond, and one (HPR) was taken off of therapy after 8 months because of disease progression after an initial response to therapy.

In both groups, there was an increase in mean hemoglobin, platelet count, and neutrophil count, coupled with decreases in mean marrow cellularity, percent marrow hairy cells, and hairy cell index with continued interferon therapy. There was a trend favoring higher hemoglobin levels in the alpha-interferon group, although perhaps due to small numbers of patients and continued transfusion in the beta-interferon group, there was overlap of 95% confidence intervals throughout therapy.

Toxicities in both alpha-interferon and beta-interferon patients included fevers, chills, headache, myalgias, fatigue, and arthralgias. These flu-like symptoms occurred in all patients, improved after 4 weeks of therapy, and did not persist after 6 months of continued therapy. They occurred with similar severity in the two groups. No patients required discontinuation of therapy or dose modification because of symptoms, and these toxicities never exceeded World Health Organization (WHO) Grade II in severity. No abnormalities were noticed in liver function tests. In the beta-interferon group, erythema, induration, and tenderness developed at sites of subcutaneous drug injection that persisted for several days. Three patients had WHO Grade II lesions (one patient required dose reduction for Grade III cutaneous toxicity). As a result of injecting beta-interferon subcutaneously into the abdominal wall, red, tender lesions developed in an additional patient at these sites which eventually became necrotic. The result was 8-cm to 10-cm nonhealing, purulent ulcers that required skin graft surgery (WHO Grade IV cutaneous toxicity). This patient was not evaluable for response and is not included in the results presented. Similar symptomatic local reactions were not seen in any of the...
alpha-interferon patients. No binding antibodies to rIFN-βser were detected in any of the patients.

**Discussion**

Alpha-interferons have demonstrated efficacy in improving hematologic variables and decreasing marrow infiltration in patients with HCL. The mechanism of action of interferon in this disease is unknown, but possibilities include a direct antiproliferative effect of interferon on hairy cells, increase in natural killer cell activity, indirect action through induction or suppression of other lymphokines, and antiviral activity. Paganelli and colleagues have shown that both alpha-interferon and beta-interferon exhibit a direct antiproliferative effect on hairy cells in vitro. This observation, coupled with in vitro evidence of a lack of correlation between natural killer cell activity and tumor cell lysis by interferon in HCL, suggests a directly antiproliferative mode of action of type I interferons in this disease.

We reported the results of rIFN-βser therapy for ten HCL patients, eight of whom were evaluable for response. Our data demonstrate a clear impact of beta-interferon in this disease and are consistent with the predictions of Paganelli and colleagues based on in vitro work. This report presented our Phase II data, together with matched historical controls treated with rIFNa2b. Due to small numbers and the nonrandomized study design, conclusions regarding the relative efficacy of alpha-interferon and beta-interferon in HCL are not possible. It does appear that hematologic and bone marrow variables improve over a similar time course with these two interferons, supporting a common mechanism of action. At the doses of subcutaneous beta-interferon used in this study, we en-
countered local toxicity not seen in patients receiving conventional doses of alpha-interferon. The component of the beta-interferon preparation responsible for the local toxicity is unknown.

There was a trend in our data favoring lower hemoglobin levels in patients receiving beta-interferon, when compared with alpha-interferon historical controls. Liu and colleagues have shown that rIFN-βser inhibits erythroid progenitor cells (CFU-erythroid and BFU-erythroid) from normal marrow in vitro. These erythroid colonies are inhibited at lower beta-interferon concentrations than myeloid colonies (CFU-GM). These in vitro findings suggest that beta-interferon can suppress erythropoiesis in vivo, and this mechanism may explain our finding of more anemia and transfusion requirement in the beta-interferon group.

We conclude that rIFN-βser has activity in HCL. The optimal dose of this medication in this disease is unknown, and it is possible that at a lower dose less anemia and less local toxicity would be encountered without compromise of therapeutic benefit.

REFERENCES