Purine nucleoside analogues, such as fludarabine, are commonly used in patients with indolent non-Hodgkin's lymphomas and chronic lymphocytic leukemia (CLL) and have promising clinical activity. However, there is concern regarding the profound immunosuppressive effects of these agents, due not only to myelosuppression but also to T-cell depletion (1–6).

Purine analogues inhibit lymphocyte function by a variety of mechanisms (7). CD4+ lymphocyte counts in particular drop precipitously and remain depressed for months and sometimes for ≥1 years after therapy discontinuation (2, 8, 9). Studies of fludarabine-based combinations have been complicated by atypical infections, including fungal infections and Pneumocystis carinii pneumonia (10–12). In our own experience with fludarabine plus cyclophosphamide for previously untreated patients with indolent lymphoid malignancies, a profound lymphocytopenia was apparent in addition to a decrease in serum immunoglobulins (12). The mean absolute CD4 counts dropped from 799 cells/μL at baseline to 139 cells/μL after treatment (P < 0.001).

Interleukin-2 (IL-2) may have value in the treatment of congenital or acquired lymphocytopenia (13, 14). In vitro studies have shown that IL-2 receptors expressed on resting T cells transduce signals that promote cell survival, without committing the T cells to proliferate (15, 16). Preclinical studies have shown that treatment of T cells with IL-2 before chemotherapy or radiation therapy can have an antiapoptotic effect (15, 16). In an effort to modulate the immunosuppression caused by fludarabine-based therapy, we conducted a phase I trial of fludarabine, cyclophosphamide, and escalating doses of IL-2 for previously untreated patients with indolent
non-Hodgkin's lymphomas and CLL. We postulated that introduction of IL-2 before chemotherapy, as well as concurrent administration, would offer the greatest immunologic protection. Dosing was based on pilot experiences with IL-2 in lymphoma patients receiving purine analogues (17) and toxicity and feasibility considerations. The objective was to find a tolerable IL-2 dose capable of supporting CD4+ lymphocyte counts, thereby reducing the risk of infectious complications.

**Patients and Methods**

**Patient selection.** This phase I safety and dose-finding trial was approved by the institutional review boards and conducted at the Johns Hopkins Hospital and Walter Reed Army Medical Center. All patients gave written informed consent. Patients had a biopsy-proven, untreated, indolent B-cell malignancy with Ann Arbor stage II noncontiguous, stage III, or stage IV disease. An indication for chemotherapy must have been present as defined by the National Cancer Institute 96 criteria for CLL (18) and for disease-related symptoms for non-Hodgkin’s lymphomas. Other eligibility requirements included serum creatinine of ≤2.0 mg/dL or creatinine clearance of >30 mL/min, bilirubin of ≤2.0 mg/dL, unless tumor-related, adequate cardiac function, and absence of active infection.

**Treatment plan.** Human recombinant IL-2 (Proleukin, Alexleukin, Chiron Corp., Emeryville, CA) was given on days 1 to 21 s.c. every 28 days and was combined with fixed dose cyclophosphamide (600 mg/m² i.v., day 8) and fludarabine (Berlex Laboratories, Seattle, WA; 20 mg/m² i.v., days 8-12) using the identical schedule previously published by our group (12). The IL-2 dose was escalated in cohorts of four to six patients, with one placebo per cohort (cyclophosphamide, antiemetics were not permitted. Patients could proceed to blood or marrow transplantation after four cycles or upon disease progression. Unless early blood or marrow transplantation was planned, patients were treated until best response or a maximum of six cycles.

Flow cytometry for CD4 cells was done at baseline, days 14 and 28 of cycle 1, at end of therapy, and every 3 months for up to 1 year. Infectious complications were monitored during therapy and for up to 1 year afterward.

The physicians, investigators, and patients were blinded to the IL-2 treatment assignment. The placebo group served principally to minimize bias in nonhematologic toxicity assessments and secondarily served as a concurrent efficacy control. Criteria for halting IL-2 dose escalations were intolerable side effects or target efficacy, defined as maintaining nadir CD4 counts of ≥500 cells/µL in at least three of four subjects and ≥350 cells/µL in the fourth subject.

Hematologic toxicities were graded according to the National Cancer Institute criteria (18). Other toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria. Fatigue was also evaluated serially with a European Organization for Research and Treatment of Cancer symptom scale (19). Responses were defined using the International Working Group criteria (20).

Dose modifications of fludarabine and cyclophosphamide were mandated for grade ≥2 toxicities and for infections requiring hospitalization. IL-2 was to be stopped temporarily if there was clinical suspicion for sepsis but restarted following control of the infection.

**Statistical methods.** Outcome variables were defined as percentage of baseline CD4 counts. Log transforms were then applied to adjust for skewness. Exploratory plots were used to describe the change in CD4 counts over time during and after treatment for each dose level of IL-2. We compared those who did not receive IL-2 to the IL-2-treated patients (dose levels 1-4) first by estimating means and confidence intervals. Linear longitudinal models were also used to assess whether there were differences across dose levels in trajectories of CD4 counts over time, both during and after treatment. Random effects models were used to account for correlation of observations taken from the same individuals. Models excluding dose were compared with models including dose (both main effects of dose and interactions between dose and time). For the model of cell behavior during treatment, time was modeled using a spline, with a knot at 40 days. For time after treatment, a linear association was assumed between time and cell counts. To determine if there were significant dose effects, Bayesian information criteria (BIC) was used, which is a standard model comparison approach for linear mixed-effects models (21).

**Results**

Twenty-three patients were enrolled on this study, 19 of whom received IL-2. The median age was 50 years (range, 41-62), and 18 patients (78%) were male. The most common histologic subtypes were CLL/small lymphocytic lymphoma (n = 7 patients, 30%) and follicular lymphomas (n = 12 patients, 52%). Other histologies were lymphoplasmacytic lymphoma/Waldenstrom’s macroglobulinemia (n = 2), marginal zone lymphoma (n = 1), and mantle cell lymphoma (n = 1).

A median of four chemotherapy cycles was given (range, 2-6). Of 17 evaluable patients in the IL-2 arm, 12 patients (71%) achieved complete remission or unconfirmed complete remission, four patients reached partial remission, and one patient had stable disease. Three patients in the placebo arm were evaluable and achieved complete remission.

**Toxicities.** Four patients ended the study after the fourth cycle because blood or marrow transplantation was planned. Reasons for completing fewer than four cycles were hematologic toxicity (n = 3) and mental status changes and depression potentially from IL-2 (n = 1). IL-2 was however generally well tolerated without dose-limiting fatigue. One patient stopped IL-2 (dose level 4) during cycle 1 due to grade 3 local skin toxicity and was considered inevaluable for response; urosepsis later developed on fludarabine/cyclophosphamide alone. Two other severe infections were documented, both on the IL-2 arm: a fatal streptococcal infection (after two chemotherapy cycles) complicating treatment of hemolytic anemia with steroids, and a cellulitis (~6 months after therapy completion) that resolved with i.v. antibiotics. No P. carinii pneumonia, herpes zoster, or disseminated fungal infections were documented.

Treatment-related toxicities were mainly hematologic, with grade 3 or 4 hematologic toxicities noted in two of four patients in the placebo arm and 7 of 19 patients (37%) who received IL-2. There was a significant correlation between the nadir CD4 count in the treatment period and the development of grade 3 or 4 hematologic toxicities (P = 0.04, Wilcoxon rank sum test).

Of 15 IL-2 patients evaluable for thyroid dysfunction, one developed a subclinical decrease in thyroid-stimulating hormone to below-normal then low-normal values, associated with a low T₄ and T₃.

**Immunologic endpoints.** Figure 1 shows, for each IL-2 dose level, the behavior of CD4 counts over time as a percentage of baseline values. The solid lines show trajectories during treatment, and the dotted lines represent behavior after study treatment has ended. Minimal difference is seen in CD4 counts during treatment across IL-2 doses as well as minimal difference after treatment. This was confirmed via longitudinal linear
models, where models of trajectories during treatment found little difference based on BIC statistics (BIC difference of 74.7 with a difference of 12 degrees of freedom, in favor of a model with no dose effects versus a model that allowed for differences in CD4 trajectories over time by dose; ref. 22). For models evaluating differences after treatment, there was also little evidence of a dose effect based on BIC (a difference in BIC of 52.4 with a difference of 8 degrees of freedom, favoring a model with no dose effects). However, the power to detect these differences is relatively low especially for testing differences after the end of therapy.

Formal statistical comparisons between the groups treated with IL-2 versus placebo were not planned. For the entire cohort, the mean absolute CD4 count was 1,008 cells/μL pretreatment (range, 97-3,776), 314 cells/μL (range, 45-2,599) at day 14, 374 cells/μL (range, 17-1,390) at day 28, and 100 cells/μL (range, 17-291) at the end of treatment.

For the evaluable IL-2 cohort, the mean absolute CD4 count was 999 cells/μL pretreatment (range, 97-3,776), 379 cells/μL (range, 54-2,599) at day 14, 376 cells/μL (range, 17-1,390) at day 28, and 98 cells/μL (range, 17-291) at end of treatment (Table 1). Nine patients in the IL-2 cohort had CD4 counts recorded at 6 months following treatment completion, with a mean of 181 cells/μL (range, 65-462).

For patients in both the placebo and IL-2 groups, all those who had an initial drop in CD4 counts to ≤20% of baseline had a subsequent increase in CD4 counts by day 28 (Fig. 1). We attribute this to hematologic recovery during the rest period.

The means of CD4 counts as a percentage of baseline at six serial time points are shown in Fig. 2 for the IL-2-treated (●) and placebo (○) groups with 95% confidence intervals. Based on this figure, there is little evidence that the IL-2-treated patients as a whole (i.e., patients on dose levels 1-4) had differences in CD4 count changes from baseline throughout the course of the study compared with the placebo group.

**Discussion**

Herein, we have shown as part of a double-blind, randomized phase I dose escalation study of IL-2 that this agent does not protect against CD4 lymphocytopenia induced by administration of fludarabine and cyclophosphamide. Specifically, at all doses of IL-2 tested including those previously shown to be beneficial in reducing lymphocytopenia induced by 2-chlorodeoxyadenosine (17), no diminishment in CD4 lymphocytopenia was noted. IL-2 therapy was generally well tolerated and did not seem to accentuate the toxicity of fludarabine and cyclophosphamide.
Previous studies have shown that IL-2 can promote survival of resting T cells (15). Addition of IL-2 to lymphocyte culture before or after a cytotoxic insult can protect against apoptosis and is associated with \(BCL2\) induction (15, 16, 23). Rescue from radiation-induced cell death by IL-2 has been shown in studies of human peripheral blood–derived T cells as well as in animal models (15, 23).

Low-dose or intermediate-dose IL-2 has been investigated as an immunostimulatory agent in patients with lymphoma (17, 24–29). The addition of IL-2 to rituximab has been well tolerated (28) and has been found to enhance antibody-dependent cellular cytotoxicity (29). A trial of IL-2 (1 \(\times\) 10⁸ IU/m²/d) administered immediately after autologous blood and marrow transplantation for lymphoid neoplasms was, however, limited by hematologic toxicities (24). Pilot results of low-dose IL-2 to prevent immunosuppression in four CLL patients with lymphocytopenia and recurrent infections after 2-chlorodeoxyadenosine have been reported (17). Here, IL-2 at

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NOTE: Dose levels 1 to 4 correspond to 0.8, 1.0, 1.2, and 1.4 \(\times\) 10⁸ IU/m²/d of IL-2, respectively. Excludes one inevaluable patient assigned to IL-2 dose level 4.
a fixed dose of $1.6 \times 10^8$ IU daily for 6 weeks was given s.c. between 2-chlorodeoxyadenosine cycles. An increase in CD4+ lymphocytes, CD8+ lymphocytes, and natural killer cells was seen, and the number of infectious complications declined. Other experiences with IL-2 in the setting of purine analogue therapy seem promising but have only been presented in abstract form (25, 26).

Although the dose of IL-2 in the above pilot trial by Dmoszynska et al. (17) is comparable with the lower dose levels of IL-2 used in our study, we did not observe protection from CD4 lymphocytopenia. Indeed, the magnitude of drop in absolute CD4 counts is similar to that in our previously published trial of fludarabine plus cyclophosphamide in the frontline setting (12). Reasons for discordant results between that pilot and our own study are potentially many. The fludarabine and cyclophosphamide combination regimen is synergistically cytotoxic and may activate pathways of lymphocyte apoptosis that are not antagonized by IL-2 administration. Alternatively, either the schedule, mode of administration, and/or dose may not have been optimal to prevent the lymphocytopenia (30). Future alternative strategies should be considered for these patients to minimize the cellular immunosuppression associated with purine analogues.

Acknowledgments

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References

Phase I Study of Low-Dose Interleukin-2, Fludarabine, and Cyclophosphamide for Previously Untreated Indolent Lymphoma and Chronic Lymphocytic Leukemia


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