

A Phase I Study of Ultra Low Dose Interleukin-2 and Stem Cell Factor in Patients with HIV Infection or HIV and Cancer

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Abstract Purpose: Ultra low doses of interleukin-2 (IL-2) can activate the high-affinity IL-2 receptor constitutively expressed on CD56^{bright} natural killer (NK) cells, the CD34⁺ NK cell precursor, and CD4⁺CD25⁺ regulatory T cells (Tregs) *in vivo*. We have previously shown synergy between IL-2 and stem cell factor (SCF) in the generation of CD56^{bright} NK cells from CD34⁺ hemopoietic progenitor cells *in vitro* and showed synergistic NK cell expansion in an *in vivo* preclinical model. To determine the safety, toxicity, and immune modulation of this combination of cytokines *in vivo*, we conducted a first-in-man phase I study.

Experimental Design: A phase I dose escalation study was conducted using IL-2 at 900,000 or 650,000 IU/m²/d for 8 weeks with 5 or 10 µg/kg/d of SCF given thrice a week for 8 weeks in patients with HIV infection and/or cancer.

Results: Ten of 13 patients completed therapy; four experienced the dose-limiting toxicities of grade 3 fatigue or urticaria. The maximum tolerated doses of IL-2 and SCF in combination is 650,000 IU/m²/d of IL-2 and 5 µg/kg/d thrice a week of SCF. NK cells were expanded over 2-fold on therapy; Tregs were expanded nearly 6-fold from baseline.

Conclusions: Administration of IL-2 with SCF is safe and well tolerated and leads to expansion of lymphocyte subsets in patients with HIV or HIV and cancer; however, the changes in NK cell and Treg expansion seen with this cytokine combination were no different than those seen with a similar dose of IL-2 alone.

Natural killer (NK) cells are large granular lymphocytes that are important in the early innate immune response to infection and malignant transformation (1, 2). Genetic disruption studies in mice and *in vitro* studies in humans show that

interleukin-15 (IL-15), flt3 ligand, and c-kit ligand or stem cell factor (SCF) are important in NK cell development (3–6). Exogenously given IL-2, using the β and γ receptor (R) components shared with the IL-15R, can mimic IL-15 in NK cell differentiation. Furthermore, the CD56^{bright} subset of NK cells coexpress a high-affinity IL-2Rαβγ and c-kit, the receptor for SCF (3). SCF alone maintains CD56^{bright} NK cell survival (7) and synergizes with IL-2 in promoting CD56^{bright} NK cell proliferation (3). We initially reported on the prolonged administration of ultra low dose IL-2 in HIV patients with cancer, showing selective expansion of CD56^{bright} NK cells and an alteration in cytokine gene expression (8, 9). More recently, we described a subset of human CD34⁺ hemopoietic progenitor cells that express a high-affinity IL-2Rαβγ, which, when activated with picomolar concentrations of IL-2, differentiates into CD56^{bright} NK cells *in vitro* (10).

Regulatory T cells (Tregs) express CD4, CD25, and FOXP3, a transcription factor that is central to Treg development (11). CD25 represents one component of the heterotrimeric high-affinity IL-2Rαβγ expressed on the surface of Tregs (12). Tregs specifically inhibit antitumor T-cell responses (13–15), whereas depletion of Tregs *in vivo* has enhanced autoimmunity and antitumor response (13, 16, 17). Whether or not human CD4⁺CD25⁺ Tregs are altered *in vivo* with a short course of ultra low dose IL-2 therapy has not been addressed and could be important for patients with infection, cancer, or autoimmune diseases.

As progressive HIV carries increased incidence of infection and cancer, enhancement of innate immune effector cells, such

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M.H. Shah directed the research study. D.M. Benson, M.H. Shah, and M.A. Caligiuri wrote the article. D.M. Benson and A.G. Freud designed, analyzed, and interpreted laboratory correlates. A.K. Ferkitich did statistical modeling and analyses. B.J. Sezube and Z.P. Bernstein assisted in patient recruitment and treatment, data collection, and execution of the study. M.A. Caligiuri designed and wrote the study protocol, obtained funding for the study, edited the final version of the article, and supervised the project in its entirety.

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as NK cells, may be beneficial; however, concomitant expansion of Tregs may also occur. Here, we report the first study in patients of ultra low dose IL-2 combined with SCF and describe the safety, toxicity, and immune modulation *in vivo*.

Materials and Methods

Study design. We examined increasing doses of SCF coadministered with established ultra low doses of IL-2 in a modified 3 × 3 schema (18). With no dose-limiting toxicity, defined as any drug-related grade 3 adverse event observed in three patients treated at a specified dose level, the dose of SCF was subsequently escalated. Toxicity was assessed using National Cancer Institute Common Toxicity Criteria, version 2.0 (19).

Patient selection. Eligible adult patients must have had an AIDS-defining illness or a history of HIV and cancer. Patients were also required to have a CD4 T-cell count of $\geq 20/\text{mm}^3$, were on highly active antiretroviral therapy, and have a Karnofsky performance status of $>70\%$. Exclusion criteria included cytopenias, pregnancy/breastfeeding, and poorly or uncontrolled cardiovascular comorbidities. Additionally, patients with asthma, positive allergy tests, angioedema, anaphylactic/anaphylactoid-type event manifested by disseminated urticaria, laryngeal edema, and/or bronchospasm and those with concurrent use of β adrenergic blocking agent were excluded.

Treatment regimen. Patients self-administered s.c. IL-2 (recombinant human IL-2 provided by Chiron Corp., Emeryville, CA) daily (except Sundays) at a dose of 900,000 IU/m²/d (cohorts 1 and 2a) or 650,000 IU/m²/d (cohorts 2b and 3) for 8 weeks. SCF (recombinant-methionyl human SCF provided by Amgen, Inc., Thousand Oaks, CA) was given in clinic s.c. by a registered nurse at 5 (cohorts 2a and 2b) or 10 $\mu\text{g}/\text{kg}/\text{dose}$ (cohort 3) thrice weekly (Mondays-Wednesdays-Fridays)

followed by at least 1 hour of close monitoring for 8 weeks. SCF prophylaxis included ranitidine (300 mg orally four times daily) and certrizine (10 mg orally four times daily) 24 hours before and 60 to 90 minutes before SCF, as well as albuterol via metered dose inhaler (two puffs) given 30 to 60 minutes before each SCF dose. Certrizine was increased to 10 mg orally twice daily if any allergic symptoms developed. Toxicity was assessed using National Cancer Institute Common Toxicity Criteria, version 2.0 (19).

Laboratory correlative studies. Using flow cytometry (BD FACSCalibur, BD Biosciences, San Diego, CA), NK and T-cell compartment expansion was assessed specifically evaluating CD3-APC, CD25-PE-Cy7, CD4-FITC, and CD56-PE (all from BD PharMingen, San Diego, CA) expression. Intracellular staining for FOXP3 (phycoerythrin, eBiosciences, San Diego, CA) was done to confirm the CD4⁺CD25⁺ population as Tregs. The proportion of CD4⁺CD25⁺ T cells among all CD4⁺ T cells and CD56^{bright} NK cells among all NK cells was compared before and during therapy. Changes in other lymphocyte subsets were analyzed to provide context for subsets of interest, as well.

To put the present findings in context, an analysis of the effects of ultra low dose IL-2 was planned comparing findings of single-agent ultra low dose IL-2 therapy (1,000,000 IU/m² s.c. daily) delivered to seven HIV⁺ patients with non-Hodgkin's lymphoma in first remission in an earlier, unpublished study (AIDS Malignancy Consortium 003/CALGB 9550). Patients in this study were of similar demographically to our present study population and received a comparable dose of IL-2 over a similar treatment course. Specifically, changes in lymphocyte subsets were conducted before therapy and after about 7 to 8 weeks of treatment.

Statistical considerations. The CD56^{bright} NK cell data and the CD4⁺CD25⁺ Tregs data were natural log transformed due to the skewness in the data. A paired *t* test was done to compare the baseline values to treatment values.

Table 1. IL-2/SCF treatment and related adverse events (with possible, probable, or definite attribution of adverse effects to IL-2 or SCF)

	IL-2 dose (units/m ² /d)	SCF dose ($\mu\text{g}/\text{kg}/\text{dose}$)	Patient UPN	Grade 1-2 adverse events	Grade 3 adverse events (DLT)	Disposition
Cohort 1	900,000	None	100	Fatigue, fever, local pruritus, arthralgia, myalgia, diarrhea, URTI, eosinophilia, increased bilirubin, transaminases, and creatinine	None	Completed
			101	Fatigue, local pruritus, myalgia, eosinophilia, increased transaminases	None	Completed
			102	Fatigue, fever, local pruritus, myalgia, diarrhea, URTI, tingling, depression, eosinophilia	None	Completed
Cohort 2a	900,000	5	200	Fatigue, fever, local pruritus, myalgia, restlessness, tight throat, sweating, dyspnea, edema, increased bilirubin, transaminases, and LDH	None	Completed
			201	Eosinophilia	None	Completed
			202	Fever, local pruritus, chills, arthralgia, myalgia, eosinophilia, increased BUN/creatinine	Fatigue	Withdrew
Cohort 2b	650,000	5	203	Local pruritus, myalgia, eosinophilia, increased LDH	Fatigue	Withdrew
			204	URT, increased LDH	None	Completed
			205	Fatigue, local pruritus, eosinophilia	None	Completed
Cohort 3	650,000	10	206	Fatigue, local pruritus, arthralgia, flushing, weight gain, eosinophilia	None	Completed
			300	Dyspnea, diarrhea, increased BUN	Urticaria	Completed
			301	Erythema/pain at injection site, nausea	Urticaria, fatigue	Withdrew
			302	Myalgia	None	Completed

Abbreviations: DLT, dose-limiting toxicity; URTI, upper respiratory tract infection; UPN, unique patient number.

Results and Discussion

Patients, treatment, and adverse events

Thirteen patients (median age = 42 years; range, 33-62 years) with AIDS ($n = 8$) or HIV and cancer ($n = 5$) were enrolled at The Ohio State University Comprehensive Cancer Center ($n = 11$) or at Roswell Park Cancer Institute ($n = 2$). All patients signed Institutional Review Board–approved informed consent before treatment. Median pretherapy CD4⁺ T-cell count was 253 cells/mm³ (range, 27-490 cells/mm³). Nine patients had undetectable HIV viral load. Five patients in the study had histories of malignancy: four with non-Hodgkin's lymphoma and one with colon cancer. All patients with cancer were in complete remission at the time of the study and were at least 8 weeks past their last cancer therapy.

Ten patients completed all planned therapy with SCF and/or IL-2, whereas three patients treated with the combination of SCF and IL-2 withdrew due to grade 3 dose-limiting toxicities (summarized in Table 1). Grade 3 fatigue was observed in two patients treated on cohort 2a with 900,000 IU/m²/d of IL-2 and 5 μg/kg/dose of SCF; therefore, the dose of IL-2 was reduced from 900,000 to 650,000 IU/m²/d for 8 weeks (cohort 2b), and all three patients in this cohort completed the entire course of therapy. As shown in Table 1, two of three patients (unique patient nos. 300 and 301) dose escalated to 10 μg/kg/dose of SCF exhibited grade 3 toxicities of urticaria and fatigue on days 23 and 31 respectively, and patient unique patient no. 301 withdrew from the study. Therefore, 650,000 IU/m² dose of IL-2 and 5 μg/kg/dose of SCF were considered the maximum tolerated doses for the combination.

Immune modulation. Changes in CD56^{bright} NK cells and Tregs were analyzed after 7 weeks of therapy. Treatment with IL-2 and SCF led to a 2.2-fold increase over baseline in CD56^{bright} NK cells ($P = 0.0274$) and to a 6-fold expansion of CD4⁺CD25⁺ Tregs ($P = 0.0057$). To put these findings in context, we analyzed the effects of single-agent ultra low dose IL-2 therapy (1,000,000 IU/m² s.c. daily) delivered to seven HIV⁺ patients with non-Hodgkin's lymphoma in first remission in an earlier, unpublished study (AIDS Malignancy Consortium 003/CALGB 9550). After an average of 8 weeks of treatment, single-agent IL-2 therapy led to statistically significant, proportional increases in CD56^{bright} NK cells (1.6-fold, $P = 0.025$) and Tregs (nearly 9-fold, $P = 0.0003$; Fig. 1). Other lymphocyte subsets were not significantly changed on therapy (see Fig. 1). Within the present study, no significant differences in lymphocyte subset changes were observed according to the dose of SCF received by each cohort in the present group (data not shown).

Conclusions and implications. Our group has previously shown that prolonged administration of ultra low dose IL-2 therapy can expand CD56^{bright} NK cells in patients with HIV infection; others have recently described expansion of Tregs with substantially higher and longer dosing of IL-2 in patients with HIV infection (20). Here, we report a first-in-human study of SCF combined with ultra low dose IL-2. We show that this combination significantly expands CD56^{bright} NK cells as well as Tregs, without any unexpected toxicities. One mechanism of CD56^{bright} NK cell expansion is likely the ability of SCF to enhance IL-2- or IL-15-mediated NK cell proliferation during NK cell development from CD34⁺ hemopoietic progenitor cells (3, 5, 6), as well as the direct

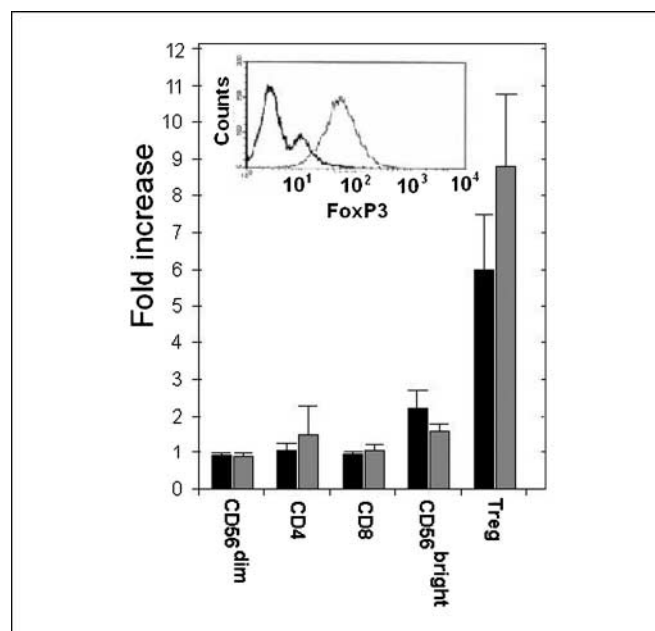


Fig. 1. Expansion of CD56^{bright} NK and CD4⁺CD25⁺ Treg cells in response to SCF and IL-2 or IL-2 alone. Fold expansion of lymphocyte subsets from pretreatment base for patients receiving IL-2 and SCF ($n = 6$, black columns) and IL-2 alone ($n = 8$, gray columns). Significant expansion of CD56^{bright} NK cells and of CD4⁺CD25⁺ T cells is seen. Inset, FOXP3 staining on the expanded CD4⁺CD25⁺ cell population from a representative patient to confirm Treg phenotype.

effect of SCF on mature CD56^{bright} NK cell survival (7). Despite this, review of data from a comparable set of patient treated with IL-2 alone would suggest that the addition of SCF to this regimen produces negligible immunomodulation over ultra low dose IL-2 alone. Perhaps the maximum tolerated dose of SCF is too low to promote proliferative synergy *in vivo* in humans. In addition, SCF is present in human serum and may already modulate or influence IL-2 directed proliferation. Finally, perhaps negative regulators of NK expansion (e.g., transforming growth factor- β) may also dampen synergistic proliferation *in vivo* in humans. As the CD56^{bright} NK cell is a powerful immunomodulatory subset in its provision of cytokines, such as IFN- γ , such expansion has the potential to enhance immune surveillance against infection and malignant transformation, especially in immunocompromised hosts.

The concomitant effect of Treg expansion is uncertain. Recently, Treg expansion in response to IL-2 was reported in patients rendered lymphopenic by cytotoxic chemotherapy (21). In this report, patients had received chemotherapy and subsequently were given autologous lymphocyte infusions followed by varying doses of IL-2. Data suggest that peripheral expansion as opposed to thymopoiesis was the major mechanism for increased Treg numbers. Although the doses of IL-2 used in this study were similar to that previously reported, the main intent of the current study was specifically to study the effects of ultra low dose IL-2 in combination with SCF; the first time these cytokines have been given to in combination in humans. Second, the patient characteristics were different in that all of our patients had HIV infection, did not receive recent chemotherapy around the time of the cytokine infusions, and did not receive autologous infusions

of lymphocytes. Of patients with a history of malignancy, none exhibited relapsed disease despite Treg expansion while on trial. No patients experienced progression of their HIV disease while on therapy as evidenced by a rising HIV viral load. Although Treg expansion may indeed counter any beneficial effects by

dampening antigen-specific immune activation (15, 17), Tregs have been associated with favorable markers of clinical HIV disease (22). Hence, the true contribution of IL-2-induced Treg expansion in states of immune deficiency will require further study.

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