Safety and Biological Activity of Repeated Doses of Recombinant Human Flt3 Ligand in Patients with Bone Scan-Negative Hormone-Refractory Prostate Cancer

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ABSTRACT

Purpose: **The purpose of this study was to evaluate the safety, biological activity, and feasibility of repeated doses of the dendritic cell (DC)-stimulating agent Flt3 ligand (FL) in patients with bone scan-negative hormone-refractory prostate cancer.**

Experimental Design: **Thirty-one patients with hormone-refractory prostate cancer who had elevated prostate-specific antigen (PSA) levels and negative bone scans were enrolled. Six cycles (28 days each) were planned. In the first cycle, patients were randomized to FL or placebo. All patients received open-label FL during the next five courses. DC, anti-FL antibody, and PSA levels were measured every 15 days to assess biological activity.**

Results: **DCs increased markedly in FL-treated patients from precycle to day 15, and the increase was consistent in each cycle. Mean percentages of DCs in peripheral blood ranged from 1.4% to 1.9% precycle and from 10.1% to 13.9% on day 15, and after the first cycle, absolute counts on day 15 were approximately 29-fold higher than precycle levels. Natural killer cell counts ($CD56^+$) were found to be elevated after cycle 1 (154% increase *versus* 2.8% decrease in placebo group at day 22). Twenty-two of 27 patients tested developed nonneutralizing anti-FL antibody. The most frequently experienced toxicity was injection site reaction, followed by asthenia, rash, and diarrhea. Although median PSA levels did not vary during any cycle, a significant slowing in velocity of PSA was observed while patients were on-study (relative velocity = 0.002) compared with prestudy PSA velocity (relative velocity = 0.007).**

Conclusions: **FL was well tolerated. FL consistently produced an increase in DC count without any evidence of decreasing response with continued exposure. The expansion of DCs and the slowing of PSA velocity after administration of FL suggest potential clinical applications in the immunotherapy of prostate cancer.**

INTRODUCTION

Recombinant human Flt3 ligand (FL) stimulates the proliferation and differentiation of hematopoietic progenitors both *in vivo* and *in vitro* by binding to and activating distinct tyrosine kinase receptors. Preclinical data have demonstrated the ability of FL to mobilize large numbers of $CD34^+$ stem cells (1, 2) and $CD56^+$ natural killer cells (3). In animal models, FL increases the number of dendritic cells (DCs) that are normal in morphology and function, based on their ability to present alloantigens in a mixed lymphocyte reaction (4). FL also produces high concentrations of circulating DCs both in healthy volunteers and in patients with metastatic colon cancer (5, 6).

DCs are the most potent antigen-presenting cells identified to date. They have the unique ability to sensitize naïve T cells to protein antigens and elicit potent antigen-specific responses (7). DCs express high levels of MHC class I and II molecules, adhesion molecules, and various costimulatory molecules on their surface. They are identified as lineage negative ($CD14^-$, $CD56^-$, $CD19^-$, $CD3^-$) and either myeloid type ($CD11c^+$, $CD123^-$) or plasmacytoid or lymphoid type ($CD11c^-$, $CD123^+$). The ability to mobilize DCs may have significant clinical impact on guiding cytolytic immune responses to destroy tumor cells (8). DCs have been successful in generating antitumor immune responses in several animal models (9–17). A variety of approaches to DC-based immunotherapy are currently under clinical investigation to evaluate whether DCs loaded with tumor antigens can be used to induce tumor immunity. Vaccination with specific tumor antigens is one setting for using large numbers of DCs. DCs are amplified *ex vivo* and then pulsed with tumor antigen and infused into a patient as a vaccine (18).

In vivo studies in mice have shown that daily injections of recombinant murine FL can produce markedly high levels of DCs in the bone marrow, gastrointestinal lymphoid tissue, liver, lymph nodes, lungs, peripheral blood, peritoneal cavity, spleen, and thymus. These cells coexpress the characteristic DC markers of MHC class II, CD11c, DEC205, and CD86. Five distinct DC subpopulations were identified in the spleens of these FL-treated mice. The cells were as efficient as the rare, mature DCs isolated from untreated mice at presenting alloantigen to T cells (4). Similarly, when FL was administered at various doses to

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healthy human volunteers, DC populations were significantly increased and consisted of both $CD11c^+/CD123^-$ and $CD11c^-/CD123^+$ populations (5). These results suggest that FL could be used to expand the numbers of DCs *in vivo*, resulting in higher levels of endogenous cells and eradicating the need to generate DCs *ex vivo*.

FL has been evaluated as an immunotherapy agent in several tumor-bearing animal models. In a mouse model of fibrosarcoma, FL administration led to complete tumor regression in 50% of animals. Biopsy of regressing tumor showed infiltration of DC-like cells. This antitumor activity was transmissible by the transfer of splenocytes (19). FL has also been shown to stimulate the proliferation of natural killer cells in mice, which may heighten an antitumor immune response (20).

With the availability of human FL, we conducted a multicenter, randomized, Phase II trial in patients with bone scan-negative hormone-refractory prostate carcinoma (HRPC) to assess the effects of repeated doses of FL over multiple cycles. The rationale for choosing prostate cancer was its association with a tumor-specific antigen [prostate-specific antigen (PSA)]. Eligible patients had elevated PSA levels and negative bone scans. The objectives were as follows: (*a*) to characterize tolerability and safety of FL; (*b*) to determine biological activity of FL by measuring DC production; and (*c*) to evaluate the effect of FL on PSA levels and progression of clinical disease.

PATIENTS AND METHODS

Patients. Eligible patients had to have a rising PSA while receiving androgen ablation therapy and must have had a negative bone scan at the time of study entry. When appropriate, a rising PSA at 4 or 6 weeks after antiandrogen withdrawal was required for flutamide and nilutamide or bicalutamide, respectively. The PSA had to be at least 5 ng/ml but less than 30 ng/ml and had to have risen on at least two consecutive occasions at least 2 weeks apart. Patients were also required to have a Karnofsky performance status of >60%, castrate levels of testosterone, adequate hematological function (absolute neutrophil count $\geq$ 1,500 cells/mm^3; platelet count $\geq$ 100,000/mm^3; and hemoglobin > 8 g/dl), adequate renal function (serum creatinine $\leq$ 2.0 mg/dl or creatinine clearance $\geq$ 50 ml/min), and adequate hepatic function (total bilirubin < 2.0 mg/dl and aspartate aminotransferase < 2× the upper limit of normal). Patients were required to wait 2 weeks for study entry if they had received colony-stimulating factor therapy or nonchemotherapy investigational agents, and they were required to wait 4 weeks for study entry if they had received prior chemotherapy or radiation therapy. Patients were excluded if they were receiving systemic corticosteroids, had a serious active infection or intercurrent illness, had a history of other malignancy, or measurable bidimensional disease. All patients gave written informed consent in accordance with federal, state, and institutional guidelines.

Treatment Regimen. The study was designed to have six 28-day cycles. Cycle 1 was a placebo-controlled, double-blind course with 1:1 randomization designed to obtain safety data. For cycle 1, 25 μg/kg FL or placebo was administered once daily for 14 consecutive days by s.c. injection given at approximately the same time each day. The dose of FL was calculated based on actual weight at enrollment and remained constant at 25 μg/kg throughout the study. For cycles 2 through 6, FL was administered to all patients once daily for 14 days by s.c. injection. No premedication was given before FL injections. All patients continued on gonadotropin-releasing hormone analog therapy throughout the study, unless they had had an orchiectomy.

After six cycles, patients could be retreated with up to four additional cycles of FL at the same dose and schedule as performed previously. To do so, they had to have stable PSA levels (PSA increase $\leq$ 25%) or a decrease in PSA levels of $\geq$50% from baseline and then a rise in PSA levels >25% (or a minimum of 5 ng/ml) after discontinuation of study drug in two consecutive determinations 2 weeks apart. During the retreatment phase, FL was discontinued if PSA levels continued to rise at the same rate.

Study Evaluations. Pretreatment evaluations consisted of a history and physical examination, performance status, and laboratory studies including complete blood count, serum chemistries, thyroid panel, urinalysis, electrocardiogram, radionuclide bone scan, PSA level, anti-FL antibody titer, DC count, and $CD34^+$ count. Precycle evaluations included physical examination, vital signs, complete blood count, serum chemistries, PSA level, and DC count. Adverse events were monitored daily; vital signs, complete blood count, DC count, and $CD34^+$ count were monitored weekly; and serum chemistries and PSA level were evaluated on day 15 of cycle 1. During cycles 2 through 6, adverse events were monitored daily, and complete blood count, serum chemistries, PSA level, and DC counts were monitored on day 15.

Toxicity and Activity Assessments. The type and grades of adverse events noted during study were tabulated and summarized. The intensity of each adverse event was graded according to National Cancer Institute Common Toxicity Criteria (21). Serum was tested by ELISA for formation of antibodies to FL. Samples that tested positive in the ELISA were tested for neutralizing antibodies defined as antibodies that block the capability of FL to bind to its receptor.

Activity was assessed by DC counts and evaluation of PSA levels. Cells with classical dendritic determinants, including $CD45^+$, $CD14^-$, and $CD11c^+$, were measured. Both absolute DC counts and percentage of DCs in the peripheral blood were enumerated. DCs were measured by fluorescence-activated cell-sorting analysis. Two PSA indices were analyzed, median change from baseline and relative velocity of PSA change.

Cell Preparation and Flow Cytometric Isolation of Peripheral Blood Mononuclear Cell Populations. The peripheral blood mononuclear cell fraction was isolated, prepared, incubated with antibodies including CD3, CD4, CD8, CD11, CD14, CD19, CD34, CD38, CD45, CD56 (PharMingen) and sorted by fluorescence-activated cell-sorting analysis as described previously (22). DCs were identified as $CD45^+$, $CD11^+$, and $CD14^-$, and monocytes were identified as $CD45^+$, $CD11^+$, and $CD14^+$. The percentage of DC was calculated from the total peripheral blood mononuclear cell count in the fraction containing Ficoll, and the DC number was the number of DCs per milliliter of blood.

Statistics. DC counts and PSA levels were analyzed using descriptive statistics. Relative velocity of PSA was defined as follows:

$$\frac{\log(y_2/y_1)}{t_2 - t_1}$$

where y_2 was the PSA value at time 2, and y_1 was the PSA value at time 1 (5). Prestudy relative velocity was compared with on-study relative velocity using a Wilcoxon signed-rank test. Statistical analysis was performed using SAS Version 6.12 (SAS Institute, Cary, NC).

RESULTS

Patient Characteristics

A total of 31 patients enrolled in this trial. The demographic characteristics of the patient population are given in Table 1. The median age was 70 years. Ninety-three percent of patients in the FL group were Caucasian, as were 88% of patients in the placebo group. For cycle 1, the mean DC percentages were 1.53% (SD, 0.98%) and 1.84% (SD, 1.23%) for the FL and placebo groups, respectively. Median pretreatment PSA levels were 15.9 ng/ml for the FL group and 20.8 ng/ml for the placebo group. Although eligibility criteria stated that patients were required to have baseline PSA levels between 5 and 30 ng/ml, six patients, who were otherwise eligible, had PSA levels above the limit and were allowed to enter the study (highest PSA level included was 49.3 ng/ml).

Doses Administered and Completion Status

A total of 151 cycles of FL were administered, with the majority of patients (90%) receiving four or more cycles of FL. In cycle 1, 15 patients received FL, and 16 patients received placebo. All 31 patients received FL during cycles 2 and 3; 21 patients completed all six cycles. Five patients continued treatment after the completion of the six scheduled cycles. The number of cycles completed and reasons for early discontinuation are listed in Table 2. Ten patients discontinued the study during cycles 4–6; six of these patients received placebo during cycle 1. Two patients discontinued due to adverse events: one experienced migraine headaches; and the other withdrew due to deep vein thrombosis.

Table 1 Demographics and baseline characteristics

Patient characteristic	FL[a] (n = 15) n	%	Placebo/FL (n = 16) n	%
Race				
Black	1	7	2	13
Caucasian	14	93	14	88
Median age (yrs)	70		71	
Range	51–88		54–82	
Median pretreatment DCs (%) (cycle 1)	1.2		1.4	
Range	1–3		1–5	
Median pretreatment PSA level (ng/ml) (cycle 1)	15.9		20.8	
Range	5–46		6–49	

[a] FL, Flt3 ligand; DC, dendritic cell; PSA, prostate-specific antigen.

Table 2 Patient completion status (n = 31)

Completion status	FL[a] n	%	Placebo n	%
No. of cycles received				
Double-blind cycle 1	15	100	16	100
Open-label cycles				
2	31	100		
3	31	100		
4	28	90		
5	25	81		
6	21	68		
>6 Cycles (no. of patients)	5			
Completed study	21	68		
Discontinued from study[b]	10	32		
Disease progression	5	16		
Adverse event	2	6.5		
Patient refusal	2	6.5		
Investigator decision	1	3		

[a] FL, Flt3 ligand.

[b] All discontinued patients withdrew during or after cycle 3; six patients who withdrew received placebo during cycle 1.

Table 3 Most common toxicities in all cycles (total n = 31)

	n	%	No. of cycles event was reported
Injection site reaction	22	71	6
Asthenia	11	36	6
Rash	9	29	6
Diarrhea	7	23	5
Pain	6	19	5
Peripheral edema	6	19	3
Nocturia	5	16	3

Safety Profile

Cycle 1. FL was well tolerated when compared with placebo in cycle 1. All adverse events that occurred were mild or moderate. The only adverse event that occurred significantly more often in the FL-treated group compared with the placebo group was injection site reactions (47% *versus* none). Three of these reactions were mild (grade 1), and four were considered moderate (grade 2); all were self-limited.

All Cycles. The most frequent toxicities occurring in five or more patients during all cycles (including placebo patients in cycle 1) are listed in Table 3. Similar to cycle 1, the most common toxicity was injection site reaction limited to grade 1 or 2 (71%). Asthenia (36%), rash (29%), and diarrhea (23%) also occurred frequently. The majority of these events were grade 1 or 2. Five patients experienced severe toxicities, including one patient each with kidney calculus, hematuria, asthenia, and deep vein thrombosis; the fifth patient had atrial fibrillation and cerebellar infarct. Of the serious events, only asthenia and deep vein thrombosis were events considered by the investigators to be possibly related to FL.

There were few grade 3 and 4 laboratory toxicities. Six

Table 4 Mean absolute counts and mean percentage change in hematopoietic progenitors and lymphocyte subsets

Cycle 1	Placebo					Flt 3 Ligand				
	Day 1	Day 15		Day 22		Day 1	Day 15		Day 22	
PBMC[a] populations	Absolute counts, cells/μl (SD)	Absolute counts, cells/μl (SD)	Mean % change, day 1 to 15 (SD)	Absolute counts, cells/μl (SD)	Mean % change, day 1 to 22 (SD)	Absolute counts, cells/μl (SD)	Absolute counts, cells/μl (SD)	Mean % change, day 1 to 15 (SD)	Absolute counts, cells/μl (SD)	Mean % change, day 1 to 22 (SD)
CD3	870 (333)	887 (343)	0.07 (20.46)	884 (358)	3.50 (17.52)	898 (403)	1046 (368)	23.01 (29.95)	1130 (435)	32.68 (33.54)
CD4	537 (224)	556 (219)	3.44 (17.69)	514 (233)	−0.18 (23.45)	533 (175)	606 (217)	18.86 (36.78)	648 (211)	28.87 (36.11)
CD8	232 (125)	201 (114)	−14.58 (21.50)	219 (133)	−0.45 (26.84)	254 (208)	300 (215)	26.96 (36.67)	316 (227)	30.38 (34.38)
CD19	204 (204)	186 (128)	5.10 (38.16)	209 (225)	48.78 (172.89)	128 (92)	182 (182)	55.53 (77.48)	166 (113)	44.88 (76.53)
CD56	175 (74)	168 (82)	−9.44 (43.93)	168 (68)	−2.81 (31.11)	172 (116)	241 (140)	78.08 (149.89)	310 (143)	154.35 (230.64)
$CD34^+38^+$	1.92 (1.27)	1.54 (1.08)	−12.78 (49.38)	1.39 (0.91)	−13.68 (58.95)	1.53 (0.79)	15.86 (13.04)	1108.57 (1198.9)	15.81 (7.78)	1000.96 (574.17)
$CD34^+38^-$	0.29 (0.44)	0.48 (0.79)	−70.07 (43.29)	0.17 (0.33)	−82.99 (38.03)	0.36 (0.33)	0.63 (0.90)	13.70 (122.62)	0.53 (0.63)	−0.84 (97.13)

[a] PBMC, peripheral blood mononuclear cell.

patients had high triglycerides, two patients had high glucose levels, and one patient each showed high bilirubin level and low sodium levels.

Biological Activity

Hematopoietic Progenitors and Lymphocyte Subset Analysis. Data for hematopoietic progenitors and lymphocyte subsets are available only for cycle 1 and are displayed in Table 4. Administration of FL increased the number of circulating $CD34^+/CD38^+$ cells by 10–11-fold starting on day 8, with a peak between days 15 and 22. Analysis of lymphocyte subsets demonstrated minor increases (<50%) in total lymphocyte counts and CD3, CD4, and CD8 counts and a 154% increase in CD56 cells in the FL group compared with the placebo group. There was no difference between groups in the number of CD19 (B) cells.

WBC, Lymphocyte, Monocyte, and DC Analysis. Baseline WBC, monocyte, and lymphocyte counts did not differ between the placebo- and FL-treated groups (Table 5). The day 15 WBC (mean ± SD) on FL or placebo was 12.89 ± 7.50 × $10^3/\mu l$ and 5.96 ± 1.35 × $10^3/\mu l$, respectively. During cycle 1, the placebo group had no appreciable change in the percentage of DCs from baseline to day 15 (mean ± SD = 1.84 ± 1.23% and 1.93 ± 1.51%, respectively). The FL group showed a significant increase in DCs (mean ± SD = 1.53 ± 0.98% pretreatment and 13.07 ± 6.01% on day 15, $P < 0.001$). DC counts before treatment and on day 15 for both groups are shown in Table 4. In the FL-treated patients, the percentage of

Table 5 Hematological and DC[a] populations

Cycle Mean (SD)	1 Placebo	1 FL	2 FL	3 FL	4 FL	5 FL	6 FL
WBC ($10^3/\mu l$)							
No. of patients Day 1/15	16/16	15/15	30/30	31/31	27/26	25/24	21/21
Day 1	6.22 (1.85)	5.83 (1.28)	6.85 (2.06)	7.42 (1.77)	7.43 (2.63)	6.95 (1.85)	7.04 (2.05)
Day 15	5.96 (1.35)	12.89 (7.50)	13.70 (6.82)	13.95 (6.49)	11.69 (5.06)	10.91 (4.52)	10.17 (3.27)
Lymphocytes ($10^3/\mu l$)							
No. of patients Day 1/15	16/16	14/15	30/29	31/31	26/24	25/24	21/20
Day 1	1.63 (0.63)	1.71 (0.70)	1.56 (0.47)	1.76 (0.62)	1.85 (0.70)	1.99 (0.75)	2.16 (0.90)
Day 15	1.51 (0.60)	1.92 (0.53)	2.41 (0.99)	2.58 (1.57)	2.19 (1.07)	2.20 (0.92)	2.25 (0.91)
Monocytes ($10^3/\mu l$)							
No. of patients Day 1/15	16/16	14/15	29/27	30/31	26/24	25/24	21/20
Day 1	0.49 (0.13)	0.50 (0.15)	0.79 (0.46)	1.10 (0.41)	1.16 (0.77)	0.94 (0.39)	0.87 (0.37)
Day 15	0.47 (0.14)	4.45 (5.07)	4.74 (3.91)	4.11 (3.11)	3.76 (2.85)	3.27 (2.20)	3.02 (1.77)
DC, no. of patients[b]							
Day 1/15	14/14	15/15	31/29	30/31	28/24	25/24	20/21
DCs (cells/μl)							
Day 1	46.84 (59.81)	22.06 (12.79)	34.52 (21.87)	51.28 (33.14)	62.93 (95.85)	52.08 (52.27)	44.64 (53.55)
Day 15	35.86 (28.10)	712.22 (450.26)	813.11 (515.81)	897.66 (847.95)	769.59 (663.76)	820.54 (1029.4)	657.49 (515.60)
DCs (%PBMC)							
Day 1	1.84 (1.23)	1.53 (0.98)	1.78 (0.99)	1.74 (0.93)	1.91 (2.39)	1.67 (0.96)	1.44 (1.41)
Day 15	1.93 (1.51)	13.07 (6.01)	13.90 (5.64)	12.77 (5.16)	13.05 (4.50)	12.61 (6.05)	10.19 (3.44)

[a] DC, dendritic cell; PBMC, peripheral blood mononuclear cell.
[b] Results were not available for two patients in the placebo group in cycle 1.

DCs on day 15 was 8.5× higher than at pretreatment. The DCs were lineage-negative $CD3^-$, $CD14^-$, $CD19^-$, $CD56^-$; $CD1b/c^+$, $CD123^-$ or $CD1b/c^-$, $CD123^+$; and they displayed low expression of CD80 and CD86 (only measured during cycle 1), indicating that they were immature (data not shown).

The biological effect of FL was consistent in all cycles in which FL was administered. The mean percentage of DCs in the peripheral blood increased markedly by day 15 of each cycle. The absolute counts and percentages of DCs are displayed in Table 5. An increase occurred during each treatment cycle, with precycle mean percentages ranging from 1.44% to 1.91%, and day 15 values ranging from 10.19% to 13.90%. After the first cycle, absolute counts on day 15 were approximately 29-fold higher than precycle levels. Corresponding increases in absolute monocyte counts were also observed over multiple cycles of FL.

PSA Levels. PSA levels did not vary markedly with any cycle. In the FL-treated group, the median pretreatment PSA was 15.9 ng/ml and the cycle 6, day 15 median PSA level was 26.8 ng/ml. However, six patients had minor decreases in PSA levels, and five patients had PSA levels that were increased by ≤25%.

The relative velocities of PSA change prestudy and during study were compared. The median relative velocity prestudy was 0.007/day (range, 0.002–0.024/day), whereas the velocity while patients were treated with FL was slower [0.002/day (range, −0.006 to 0.021/day). This difference of −0.005/day was significant ($P < 0.0001$).

Retreatment

Five patients qualified for retreatment with FL. A range of 6–13 weeks elapsed between the end of the 6-month study period and the beginning of retreatment. At baseline of the retreatment period, four of the five patients had PSA levels that were equal to or higher than those at study entry. One patient experienced a reduction in PSA levels during the retreatment period similar to his response during the 6-month study (retreatment baseline PSA = 58.2; end of retreatment PSA = 49.4). One patient had a net decrease in PSA levels between study baseline (PSA = 20.7) and the end of treatment period (PSA = 11.7), although his PSA levels increased during retreatment (retreatment baseline PSA = 20.2; end of retreatment PSA = 25.1).

Antibody Formation

Twenty-seven patients were tested for anti-FL antibody in samples obtained at baseline and the end of study (11–14 days after last dose) using an ELISA method; 22 (81%) were positive. All specimens that tested positive were subjected to an assay for neutralizing antibody, and all 22 specimens were negative.

Ten patients who tested positive for anti-FL antibody at the end of study had additional samples available from the first 2 months of the study. Four of these patients were also positive in the earlier samples. Corresponding cycle day 15 DC counts (obtained approximately 2 weeks after these antibody samples) were elevated, consistent with day 15 counts for the patients' other cycles, indicating that anti-FL antibody did not inhibit the biological activity of FL.

DISCUSSION

In this study, 31 men with HRPC and negative bone scans were given FL. Cycle 1 included a double-blind, placebo-controlled treatment arm, whereas all patients were treated with FL during the five remaining cycles. FL was found to be well tolerated. The most frequently reported toxicity was injection site reaction. Other than this self-limited, localized reaction, comparison with placebo-treated patients showed no clinically relevant differences in the incidence of toxicities. When safety data were assessed for all cycles combined, no other clinically significant adverse events were associated with administration of FL.

Although a high proportion of patients (22 of 27 patients) tested positive for anti-FL antibody at end of study, biological activity did not appear to be altered. All patients were negative for neutralizing antibodies, suggesting that the binding capacity of FL remained intact. The four patients who were anti-FL antibody positive during the first 2 months of the study had increases in day 15 DC counts consistent with other cycles.

Although overall PSA levels remained unchanged with FL treatment, 11 patients had a decrease or only a minor increase (<25%) in PSA. The median relative velocity was significantly less in patients after FL treatment. Unpublished results[6] rule out the possibility that FL inhibits PSA production by prostate tumor cells. The human prostate carcinoma cell line LNCaP was used in assays for secreted PSA, intracellular PSA, and androgen receptor and the transcriptional effects of both. In each experiment, PSA levels did not decrease significantly when the cell line was treated with FL. These results indicate that FL does not directly interfere with PSA production or result in direct cytotoxicity.

To generate an immune response to tumor cells, barriers to antigen presentation must be overcome. Generally, tumor-specific antigens elicit weak immune responses *in vivo* (23–25). DCs are the most efficient antigen-presenting cells; however, they normally make up only a small fraction of WBCs (<1%; Ref. 24). Expansion of DCs that acquire, process, and present antigen may heighten the immune response to tumor antigens. In this study, the 29-fold increase in DC numbers after FL administration approached the increase seen in FL-treated normal volunteers (10–44-fold; Ref. 5). The proliferation and expansion of DCs after FL has correlated with biological activity in animal models (4) and patients with metastatic colon cancer (6). Whereas studies are evaluating *in vitro*, antigen-pulsed DCs as antitumor therapy (18, 26–29), the procedure is labor-intensive, and there is no assurance that the DCs will migrate to lymph nodes, where antigen presentation takes place (30). The results presented here demonstrate that HRPC patients treated with FL had an *in vivo* expansion of classical DCs.

Two distinct populations of DCs have been identified: a myeloid DC ($CD11c^+$, lineage-negative, $CD123^-$) or DC1; and a plasmacytoid DC ($CD11c^-$, lineage-negative, $CD123^+$) or DC2. Although the precise role of the two DC types is still being determined, there is evidence that they may differ in their ability

[6] W. D. Figg, personal communication.

to induce naïve T helper cells to differentiate to either TH1 or TH2 cells (31). The clinical significance of the different DC types is unknown; however, FL administration in these prostate cancer patients resulted in the generation of both subtypes of DCs, as seen when FL was administered to normal volunteers (5, 32). FL is the only cytokine shown to expand both DC populations *in vivo*. The DC populations expressed low levels of CD80, CD86, and HLA-DR, indicating that they were immature DCs, which are very efficient at antigen uptake and processing (24).

An antitumor effect may also depend on expansion of effector cells. In animal studies, FL has been shown to increase the natural killer cell population (20). In this study, natural killer cell counts ($CD56^+$) were found to be elevated after cycle 1 (154% increase *versus* 2.8% decrease in placebo group at day 22).

FL administration was found to be safe and well tolerated in a previous study of FL given to healthy volunteers as a single cycle and in patients with colon cancer, where one patient received three cycles (4, 6). A significant finding in the current study was that the biological response was consistent over five consecutive cycles, with no exhaustion of DC expansion. During the last cycle of FL treatment, the percentages of DCs generated were no different from the initial cycle of FL.

In summary, FL was well tolerated by patients with bone scan-negative HRPC. This confirms the safety profile seen in other human trials. DC expansion illustrated that FL provides *in vivo* biological activity in prostate cancer. The relative velocity of PSA change during FL treatment exhibited a stable slope compared with a significantly less steep slope of the velocity prestudy. The clinical significance of this observation is unknown but warrants further study.

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