The Safety and Effect of Topically Applied Recombinant Basic Fibroblast Growth Factor on the Healing of Chronic Pressure Sores

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The first randomized, blinded, placebo-controlled human trials of recombinant basic fibroblast growth factor (bFGF) for pressure sore treatment were performed. Three different concentrations of bFGF in five dosing schedules were tested for safety using hematology, serum chemistries, urinalysis, absorption, antibody formation, and signs of toxicity. Efficacy was evaluated by wound volumes, histology, and photography. No toxicity, significant serum absorption, or antibody formation occurred. In six of eight subgroups, there was a trend toward efficacy with bFGF treatment. When all subgroups were combined, comparison of the slopes of the regression curves of volume decrease over initial pressure sore volume demonstrated a greater healing effect for the bFGF-treated patients ($p < 0.05$). Histologically, bFGF-treated wound sections demonstrated increased fibroblasts and capillaries. More patients treated with bFGF achieved >70% wound closure ($p < 0.05$). Blinded observers were able to distinguish differences in visual wound improvement between bFGF and placebo groups. These data suggest that bFGF may be effective in the treatment of chronic wounds.

AN AGENT CHEMOTACTIC for inflammatory cells, such as neutrophils or macrophages, and mitogenic for cells important in wound healing, such as fibroblasts or endothelial cells, theoretically could be of benefit in augmenting healing, particularly healing of chronic wounds. Similarly, angiogenic substances, or agents increasing collagen synthesis, could be useful. Basic fibroblast growth factor (bFGF) is a polypeptide that exhibits a wide range of *in vitro* biologic activities, such as stimulation of cell mitogenesis and chemotaxis.[1-3] The mitogenic effects of bFGF are directed primarily toward cells of mesodermal or neuroectodermal origin.[4] Because bFGF is a potent mitogen and chemoattractant for capillary endothelial cells, it has been shown *in vivo* to be an effective stimulant of neovascularization and ultimately collagen synthesis.[5,6] Recently it has been discovered that bFGF is also a mitogen for certain ectodermal cells, such as keratinocytes.[7,8]

Based on the wide range of cell types known to be bFGF sensitive, it has been hypothesized that exogenously administered bFGF should accelerate the rate at which wounds heal. With the ability to clone bFGF, large amounts of recombinant bFGF have become available for study. Hebda et al.[9] demonstrated that bFGF accelerated epidermal healing in a porcine model using partial-thickness excisional wounds. Klingbeil et al.[10] demonstrated acceleration of wound closure in full-thickness excisional wounds in the congenitally diabetic mouse. Stenberg et al.,[11] from this laboratory, showed that bFGF could overcome the inhibition to wound contraction demonstrated in acutely contaminated wounds. Each of those animal trials demonstrated a marked effect in the aspect of healing under study using a single application of bFGF. Hayward et al.[12] used a model more closely related to chronically contaminated human wounds. In this model, repeated applications of bFGF accelerated closure of a chronically contaminated wound. Based on these studies, it was postulated that recombinant bFGF might be beneficial in the treatment of chronic indolent human wounds.

The purpose of the current study was to perform the

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TABLE 1. *Study Design*

Group	Application Schedule (days) 1	4	7	10	13	16	19	22	N
Tier 1: Low-dose bFGF (1.0 μg/cm²)									
A	GF	V	V	V	GF	—	—	—	4
B	GF	GF	GF	GF	GF	—	—	—	4
C	GF	GF	GF	GF	GF	GF	GF	GF	3
$Placebo_1$	V	V	V	V	V	—	—	—	3
Tier 2: High-dose bFGF (10.0 μg/cm²)									
D	GF	V	V	V	GF	—	—	—	4
E	GF	GF	GF	GF	GF	—	—	—	4
F	GF	GF	GF	GF	GF	GF	GF	GF	3
$Placebo_2$	V	V	V	V	V	—	—	—	3

Tier 3: Intermediate-dose bFGF (5.0 μg/cm²)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
G	GF	GF	GF	GF	GF	GF	GF	GF	GF	GF	GF	GF	GF	GF	GF	GF	GF	GF	GF	GF	GF	7
$Placebo_3$	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	3
H	GF	GF	GF	GF	GF	—	GF	—	—	—	—	—	—	GF	—	—	—	—	—	—	GF	6
$Placebo_4$	V	V	V	V	V	—	V	—	—	—	—	—	—	V	—	—	—	—	—	—	V	6

GF, bFGF; V, placebo vehicle. H and P_4 were performed at UNC.

first human trials to demonstrate the safety and effect of topically applied recombinant basic fibroblast growth factor in chronic wounds. The type of wound chosen for study was the pressure sore.

Methods

Randomized, blinded, placebo-controlled trials were performed on 50 patients with pressure sores extending from the bone to the subcutaneous tissue (grades III/IV). All patients were denervated in the area of ulceration because of either congenital or acquired spinal cord pathology. The specific growth factor used in these trials was recombinant bFGF produced in *E. coli* with a molecular weight of approximately 18 kd and an isoelectric point of 9.8. The investigational protocol was approved by Institutional Review Boards of The University of Texas Medical Branch and the University of North Carolina.

Consecutive patients, fulfilling entry criteria and having an ulcer between 10 and 200 cm^3, were randomized to receive either a placebo or recombinant bFGF. Patients received bFGF at concentrations of 100 μg/mL, 500 μg/mL, or 1000 μg/mL according to different schedules for periods up to 22 days, depending on the tier of the study (Table 1). The test material was calculated based on a dose volume of 0.01 mL/cm^2 of ulcer surface. Evaluation of the acute treatment phase lasted for a total of 30 days inclusive of treatment.

Sequential dose escalation was performed with unequal allocation of patients to the recombinant bFGF or placebo treatment at each tier (Table 1). After demonstration of safety at a single site (University of Texas Medical Branch), the additional institutional site (University of North Carolina) was added for the final tiers.

Patients meeting the study inclusion/exclusion criteria (Table 2) underwent a screening evaluation, including a medical history, physical examination, baseline chest x-ray, and electrocardiogram before beginning the treatment. Nutritional assessment, including anthropometric measurements, total lymphocyte counts, and urine urea nitrogen, was obtained. If sharp debridement of the ulcer was required to remove any necrotic tissue, initial drug administration was delayed for at least 24 hours after the debridement.

TABLE 2. *Inclusion/Exclusion Criteria*

Major inclusion criteria
Age: 18–65 yr
Pressure sores: 10–200 cm^3 as measured by alginate mold
Hospitalized patients
Mechanical debridement (if necessary): at least 24 hr before initiation of treatment
Laboratory findings: normal or clinically insignificant abnormalities on pretreatment CBC, coagulation, chemistry, urinalysis panels
Major exclusion criteria
Arterial or venous disorder, or vasculitis as cause for ulcerated wound
Clinically significant systemic disease
Significant malnutrition
Recent use of steroidal therapy
Penicillin allergy

To determine safety, hematology, serum chemistry, and urinalysis values were determined on days 6, 12, 16, 23, and 30 after drug initiation. Any adverse reactions, intercurrent medical events, and concomitant medications were recorded daily. Serum samples were obtained on day 1 of the trial to evaluate serum absorption at various times from 15 minutes to 4 hours after application. Serum also was obtained at the time of screening and at the 1-month follow-up visit to screen for the development of antibodies to recombinant bFGF.

Measurements of the pressure ulcer were performed on days 0, 8, 16, 23, and 30 using planimetry of the ulcer opening; maximum perpendicular diameters of the surface opening and maximum depth of the crater; volume determination using alginate molds[13]; color photography of the ulcer at a set focal distance; quantitative and qualitative microbiology of wound tissue biopsies; and histologic analyses of wound tissue.

Drug application was performed according to the specific tier after irrigation of the ulcer crater with normal saline. The given drug dosage was applied from a spray applicator, after which the wound was exposed to the ambient air for 15 minutes to allow the medication to adsorb to the wound surface. After this time, the ulcer crater was packed with fresh saline-moistened sterile gauze. Twelve hours later the saline-moistened gauze was changed, but no additional medication was applied.

Standard pressure-relieving devices were used as appropriate, and their use was documented. Patients not on air-fluidized beds were repositioned rigorously at 2-hour intervals throughout the treatment period. Wound biopsies were obtained weekly for bacterial and histologic analyses. The biopsies for histology were evaluated in a blinded fashion by a single pathologist.

On completion of the 30-day acute evaluation period, patients were discharged with follow-up evaluations at 1, 3, and 5 months. Those subjects with any remaining wound opening had wound measurements taken as during the acute trial. Ulcers that had healed or had been surgically closed during the follow-up period were evaluated for quality of scarring as well as for possible recurrence.

Descriptive statistics were computed for demographic characteristics such as age, gender, ethnicity, and pressure sore duration. The patients' ages and sore durations were compared using the Wilcoxon two-sample test, whereas gender and ethnicity were compared using the Fisher's exact test.

Both parametric and nonparametric analyses were used to determine efficacy of bFGF, depending on the apparent normality of the data. Percentage decrease in volume over 30 days was compared in each bFGF dosage regimen patient group with the placebo-treated patients, using analysis of variance. To assess for response rate relationships to initial pressure sore size, actual decrease in volume was compared with initial wound size and regression analyses were performed. The slopes of the regression curves then were compared with the F test.

Because previous trials with the pressure sore model used in this study showed a placebo response of up to 50% decrease in volume, and a topical antimicrobial response of 60% reduction over a 4-week period,[14] an arbitrary response rate of 70% wound closure over 30 days was chosen as indicative of a responder. Categorical responders by this definition were compared between bFGF-treated patients and placebo-treated patients using analysis of variance.

Results

Forty-nine of 50 patients completed the 30-day acute phase of the trials at the two institutional sites. One patient was removed from the trial during the first week when further evaluation of his chest x-ray raised suspicion of a possible neoplasm. On breaking the code at the conclusion of the study, the patient had been randomized to the placebo group. Demographics disclosed no significant differences in age, gender, ethnicity, or duration of the pressure sore between patients treated with bFGF and those receiving the placebo (Table 3).

No hematologic, chemical, or urinalysis abnormalities appeared that were attributable to the topical administration of recombinant bFGF. Basic FGF was not detectable in serum samples drawn from 15 minutes to 4 hours after topical administration of the drug. Antibodies to recombinant bFGF were not detectable either during the treatment phase of the trial or during the follow-up period.

The major response examined was the percentage decrease in wound volume over the 30-day acute evaluation phase. There was no statistical difference (analysis of variance) between mean initial sizes in the placebo and bFGF-treated groups. The data showed that the actual volume decrease in milliliters that a sore exhibited, regardless of its treatment, was directly proportional to its initial size.

TABLE 3. *Demographics of Patients and Pressure Sores*

Parameter	Group	Mean ± SD or *N*	*N*	p
Age (yr)	bFGF	37.8 ± 13.2	35	
	Placebo	37.9 ± 12.8	14	NS*
Sex	bFGF	Men 30; women 5	35	
	Placebo	Men 9; women 5	14	0.124†
Ethnicity	bFGF	Caucasian 12; Black 18; Hispanic 5	35	
	Placebo	Caucasion 6; Black 5; Hispanic 3	14	NS†
Sore duration (mo)	bFGF	17.7 ± 21.6	35	
	Placebo	25.9 ± 46.3	14	NS*

* Wilcoxon two-sample test.
† Fisher exact test.

Younger patient age and shorter sore duration before commencing the trial correlated with a slightly better response to treatment. However, this effect was small, and ages and duration of the pressure sore were equally distributed between groups (Table 3).

As noted previously, there was a correlation between the actual decrease in pressure sore size and the initial wound size. This response seemed to be enhanced in six of the eight bFGF dose regimens tested, more specifically in the five highest doses administered (Fig. 1). Slopes of the regression curves were compared with the F test, with a trend for bFGF enhancing a decrease in volume of the sore as compared with controls in the six subgroups. When all patients receiving bFGF at the two institutional sites were combined as a group, the difference between the slopes of the treated and placebo curves were significant at $p < 0.05$ (Fig. 2).

No statistically significant differences were seen when percentage decreases in wound volumes over the 30-day treatment evaluation period were compared among the various dosage groups. The trend toward efficacy for bFGF was again apparent when all bFGF-treated patients were

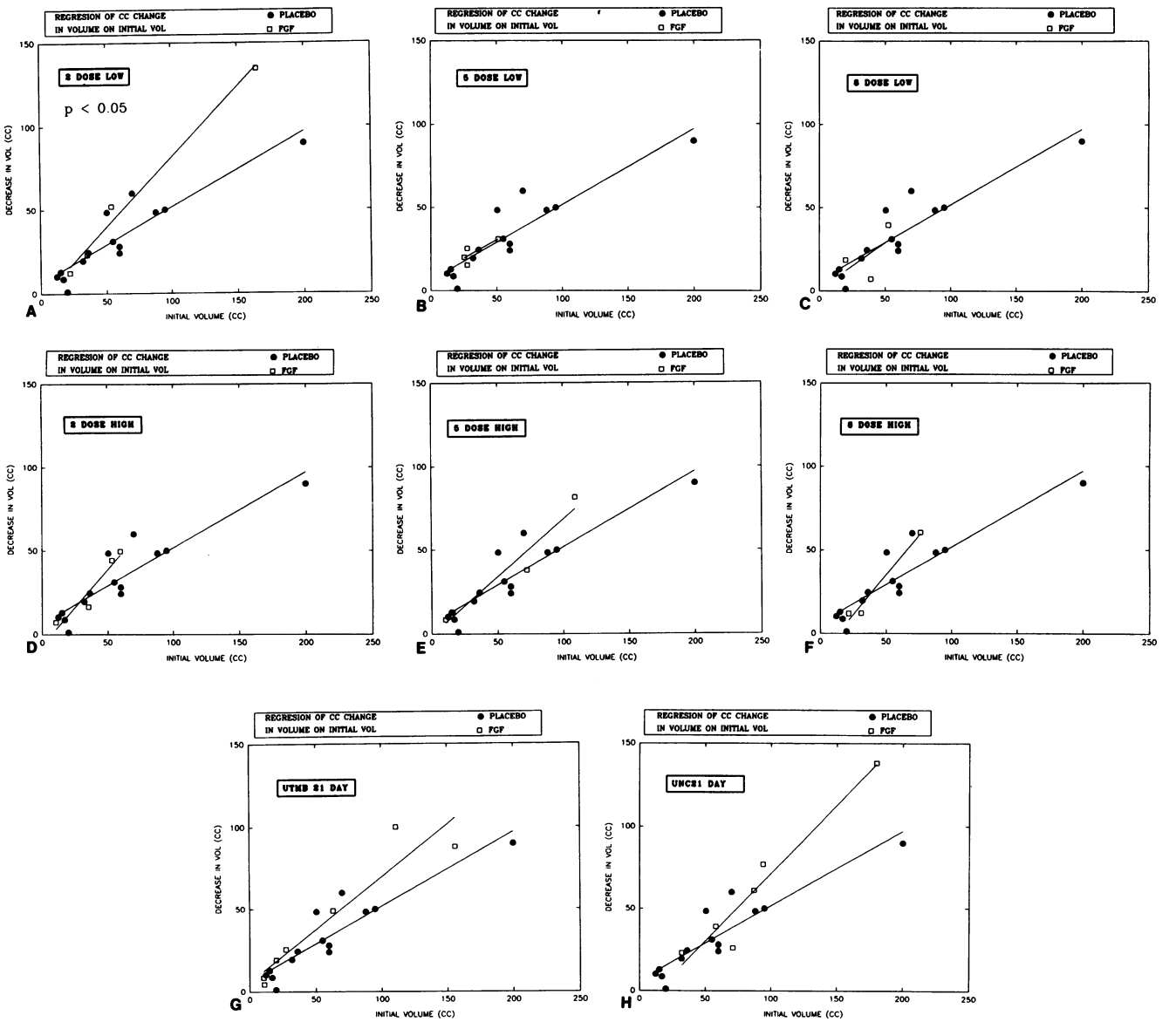

FIG. 1. Decrease in pressure sore volume over a 30-day period compared with initial volumes for bFGF-treated patients and placebo-treated patients. (A) 1.0 $\mu g/cm^2$ bFGF administered on days 1 and 13. (B) 1.0 $\mu g/cm^2$ bFGF administered on days 1, 4, 7, 10, and 13. (C) 1.0 $\mu g/cm^2$ bFGF administered on days 1, 4, 7, 10, 13, 16, 19, and 22. (D) 10.0 $\mu g/cm^2$ bFGF administered on days 1 and 13. (E) 10.0 $\mu g/cm^2$ bFGF administered on days 1, 4, 7, 10, and 13. (F) 10.0 $\mu g/cm^2$ bFGF administered on days 1, 4, 7, 10, 13, 16, 19, and 22. (G) 5.0 $\mu g/cm^2$ bFGF administered daily for 21 days. (H) 5.0 $\mu g/cm^2$ administered on days 1–5, 7, 14, and 21.

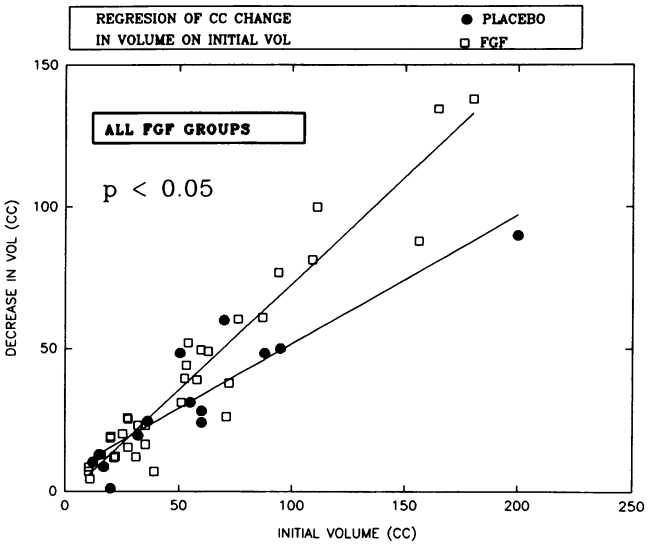

FIG. 2. Decrease in pressure sore volume after a 30-day period compared with initial volume for all patients treated with bFGF and the placebo vehicle. Slopes of the regression curves are statistically different when compared with the F-test ($p < 0.05$).

compared with the controls. Here the mean percent decrease in volume was 69% for the bFGF-treated wounds, compared with 59% for the placebo response.

When the data were analyzed in terms of the number of patients achieving a 70% volume reduction, 21 of 35 patients receiving bFGF responded, *versus* four of 14 patients in the placebo group (Table 4). This outcome is significantly different when analyzed by the Fisher's exact test ($p = 0.047$). Interestingly, a similar response is seen among the two groups treated with bFGF for 21 days (six at University of North Carolina and seven at University of Texas Medical Branch). Of the 13 patients, nine achieved >70% closure ($p = 0.041$).

Histologically, bFGF produced a marked increase in fibroblasts and capillaries (Fig. 3). Evaluation of the standardized color photographs of the pressure sores taken at day 30 allowed blinded observers to distinguish significant differences between the bFGF and placebo-treated wounds in visual improvement of the ulcer overall. The observers determined a difference in the global response to treatment and the degree of epithelization in the bFGF-treated sores as compared with the controls.

Discussion

Pressure sores are chronic wounds with an estimated 3% to 5% incidence in hospitalized patients.[15-18] This incidence has been reported to increase to 25% to 85% in patients with spinal cord injuries.[19] Assuming that 5% of the approximately one million Americans hospitalized annually will develop pressure sores, and using the 1977 estimate of $15,000 for cost of care per patient,[19] the total

TABLE 4. *Response of >70% Decrease in Volume in 30 Days*

	Responders (%)	Nonresponders (%)
bFGF	60	40
Placebo	29	71

* $p = 0.047$, Fisher exact test.

cost of treatment is a staggering $750,000,000 per year, not accounting for inflation.[15]

More rapid healing of a chronic wound is significant because it could result in decreased hospitalization and earlier return to function. In a small trial completed by the senior author on recombinant PDGF-BB, it appeared that healing rates could be improved using topically applied growth factors.[20] In our institution, care of such chronic wounds currently costs approximately $1,000 per day. Recombinant epidermal growth factor has been reported by Brown et al.[21] to speed healing of split-thickness graft donor sites. They also suggested accelerated healing in a mixture of chronic ulcerated wound types.[22] Therefore, the current trial reporting the first human use of recombinant bFGF is the third effort in which topically applied growth factors have been evaluated for their ability to accelerate wound healing.

The small number of patients in each of the various dosage regimens in this study prevents complex statistical analyses. Lack of significance for the trends reported here should not be overinterpreted. Small stepwise increases in bFGF administration were necessary for safety evaluation because this was the first trial for this agent on humans. Histologic differences were apparent, even within the subgroups of patients. When numbers of patients were increased by lumping the bFGF-treated patients, an apparent effect was seen in the increased volume reduction compared with initial pressure ulcer size, by histology, and by evaluation of epithelization and global response as determined by standard photograph examination by blinded observers.

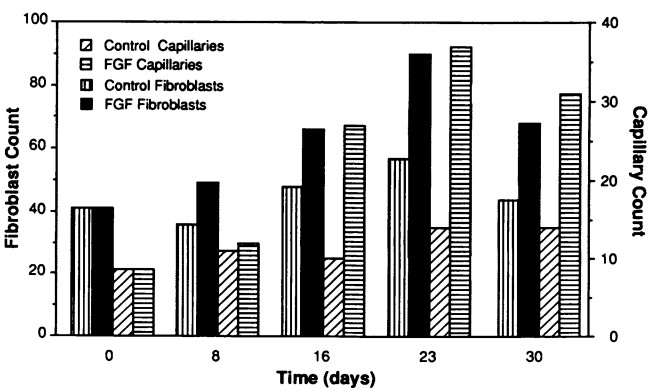

FIG. 3. Fibroblast and capillary counts from wound sections obtained from patients treated with either bFGF or placebo vehicle.

The results of these trials are very similar to those reported from our laboratory for contaminated animal wounds. In both the acutely contaminated excisional wound reported by Stenberg et al.[11] and the chronically contaminated granulating wound reported by Hayward et al.,[12] bFGF appeared to overcome the inhibition to wound contraction caused by bacteria. The exact cause for indolence in pressure sores is not known. It has been suggested that tissue bacterial levels may inhibit wound healing.[23] If this is the case, the pressure sore would be very similar to the bacterially inhibited animal models.

Demonstration that a peptide growth factor may speed healing in the chronic pressure sore is important. Recent data show that operative ablation of the ulcer with reconstruction is not universally successful.[24] In many patients, this operative approach is not possible, either because of the overall health status of the patient or because of repetitive breakdown having exhausted all possibilities of reconstruction. Even when surgical approaches are initially successful, 61% of pressure sores recur within 9.3 months.[24] This incidence increases to 79% in patients with traumatic paraplegia, the most frequent diagnosis of the patients in this study.

Although the numbers are small in the various tiers, this first human trial suggests that topically applied recombinant bFGF is safe, and may be effective in the treatment of chronic wounds such as pressure sores.

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Discussion

DR. JOHN A. MANNICK (Boston, Massachusetts): I congratulate Dr. Robson on conducting a prospective and well-carried-out study to try to determine whether these new biologic growth factors really are going to be of help clinically. Dr. Robson was kind enough to send me a manuscript even though he knows I know absolutely nothing about this field. I am really here because Judah Folkman, who does, could not attend this meeting and I guess I was the closest colleague Marty could find.

The reaction I have to this study is that it clearly shows a trend. Because this whole field is so mired in controversy, having a lot to do with the money that is going to be made if these things work, do you think the