A Randomized, Double-Blind, Placebo-Controlled Trial of Transfer Factor as Adjuvant Therapy for Malignant Melanoma

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One hundred and sixty-eight evaluable patients participated in a randomized, double-blind study of transfer factor (TF) *versus* placebo as surgical adjuvant therapy of Stage I and Stage II malignant melanoma. Eighty-five patients received TF prepared from the leukocytes of healthy volunteer donors; eighty-three participants received placebo. Therapy was initiated within 90 days of resection of all evident tumor and continued until 2 years of disease-free survival or the occurrence of unresectable dissemination of melanoma. Known prognostic variables were similarly distributed in the treatment and control groups, documenting the randomization efficacy. Three endpoints were analyzed: disease-free interval, time to Stage III metastasis, and survival. After a median follow-up period of 24.75 months, there was a trend in favor of the placebo group with regard to all three endpoints and this was significant ($P \leq 0.05$) for time to Stage III metastasis. These findings indicate that TF is not effective as surgical adjuvant therapy of malignant melanoma.

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DETERMINATIONS OF primary tumor thickness and level, in conjunction with other factors of disease stage, patient sex, and primary tumor location, make possible predictions of considerable accuracy regarding the eventual course of malignant melanoma.[1] Beyond surgical removal of obvious tumor, however, little can be done to improve the prognosis for patients at high risk for tumor recurrence.[2] Adjuvant chemotherapy has proved ineffective and has demonstrated considerable toxicity.[3,4] Adjuvant radiation therapy has a limited role in melanoma management.[5] As a result, emphasis has continued to be placed on the development of an effective, nontoxic, therapeutic adjunct to surgery in order to suppress the growth of the residual, microscopic tumor metastases which account for disease recurrence and death.

Malignant melanoma has been considered a disease well suited to immunotherapeutic intervention based on the postulate that it represents a particularly immunogenic tumor. Histologic regression within primary melanomas has been noted to occur in as many as 16% of cases[6]; an associated dense lymphocytic infiltrate is often present within regressing tumors.[7] Spontaneous regression of widespread disease has also been documented,[8] it having been estimated that melanoma accounts for 15% of reported regressions of malignancies, though it represents only 1% to 3% of cancers.[9] The regional waxing and waning of subcutaneous melanomas in patients with limited disease, and the extremely slow progression of disease in certain individuals is well known.[10] Though proof of immune mechanisms has been lacking, these unusual but significant findings have been attributed to host immune responsiveness or changes in host immunologic status (brought on by concurrent infection, for example[8]). Further support for immunotherapy of melanoma is provided by the intradermal and lymphatic locations of the disease, locations ideal for attempts at cellular immune modulation.

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Transfer factor (TF) is a dialyzable extract from the disrupted peripheral lymphocytes of an antigen-sensitized donor which can passively confer cell-mediated immunity upon an unimmunized recipient. First described in 1955,[11] TF has since been characterized as a low molecular weight (less than 12,000 daltons), nonimmunogenic substance with both antigen-dependent and -independent actions on macrophages and lymphocytes.[12] Attempts to employ these effects practically has led to the use of TF as an antitumor agent. Our preliminary studies suggested that transfer factor might be effective as adjuvant therapy for melanoma patients,[13] including those with surgically resectable lung metastases.[14] Its adjuvant use also was supported by the favorable results of a nonrandomized, historically controlled trial of transfer factor in high-risk Stage I melanoma, reported by Blume and coworkers.[15]

These considerations led us to undertake a randomized, double-blind, placebo-controlled trial of TF as adjuvant therapy in malignant melanoma treated surgically.

Patients and Methods

Patient Eligibility

Patients participating in the study had histologically documented malignant melanoma and prognostic indicators suggesting a high risk for disease recurrence. For patients with Stage I disease inclusion criteria were primary melanoma with a Clark's level of III, IV, or V and a thickness of 1.25 millimeters or more; mucosal, subungual, or acral-lentigenous melanoma; or regional cutaneous or subcutaneous recurrence of disease. Patients with Stage II disease who had histologic confirmation of regional lymph node metastasis were likewise eligible. Patients with Stage III melanoma were excluded from study.

The patients' referring physicians provided appropriate surgical treatment before protocol entry, *e.g.*, wide reexcision of the primary lesion, resection of a local or regional recurrence and/or regional lymph node dissection. Radiotherapy or isolated limb perfusion were employed, when necessary, to ensure clinical disease-free status prior to study entry. In so far as possible, all primary melanoma histologic material was reviewed by a single dermatopathologist. After referral to the Melanoma Clinic, confirmation of clinical tumor-free status was documented by physical examination, complete blood count, serum chemistry panel (with liver enzyme determinations), urinalysis, radionuclide scan of the liver and spleen or abdominal computed tomography (CT), and either whole-lung tomography or chest CT scan. Administration of the assigned medication had to begin within 90 days of the last surgery demonstrating positive histologic evidence of melanoma. This 90 day limit allowed time for patient referral, Melanoma Clinic evaluation, and patient staging in the postoperative period.

Staging, Stratification, and Randomization

The melanoma staging system accepted by the International Union Against Cancer was used for patient classification. Stage I patients were defined as those with only regional melanoma, *i.e.*, primary lesions, local cutaneous or subcutaneous metastases, or "in transit" skin metastasis greater than 5 cm from the primary lesion but not yet spread to regional lymph nodes. Stage II patients had regional nodal tumor involvement or lymph node disease without a known primary melanoma. Patients with melanoma dissemination beyond the regional lymphatic drainage, to cutaneous, subcutaneous, distant nodal, or visceral sites were considered to have Stage III disease and were not eligible.

Patients were stratified, depending upon clinical presentation, into one of the following five patient classes:

1. Primary melanoma Clark's level III, IV, or V, 1.25 mm or greater in thickness, with no node dissection (clinical Stage I).

2. Primary melanoma Clark's level III, IV, or V, 1.25 mm or greater in thickness, with node dissection negative (histologic Stage I). Volar, subungual, or mucosal primary melanoma with no lymph node dissection or node dissection negative (clinical or histologic Stage I).

3. Primary melanoma Clark's level III, IV, or V, 1.25 mm or greater in thickness, with node dissection positive within 90 days of primary melanoma biopsy (histologic Stage II).

4. Node dissection positive greater than 90 days from primary melanoma biopsy (clinical and histologic Stage II). Nodal metastatic melanoma of unknown primary (clinical and histologic Stage II).

5. Isolated limb perfusion or radiation required after surgery to ensure elimination of all evident disease (clinical or histologic Stage I or II).

There was no further stratification; other factors important in determining prognosis, *e.g.*, sex, age, and primary tumor thickness, were reserved as covariates in the analysis of results.

Randomization within each class was done in balanced sets of six patients to ensure approximately equal numbers of participants in either the TF or placebo study arms. Neither study participants nor the investigators knew the nature of the individual medication assignments, whether TF or placebo, during the course of the trial.

Protocol

Study participants received either a three milliliter (5×10^8 lymphocyte equivalents) subcutaneous injection of TF or three milliliters of placebo. Injections were administered either at the Melanoma Clinic or by the patients' primary physicians under the direction of the principal investigators. Injections were given every three weeks. This schedule was chosen based on past experience demonstrating that skin test reactivity following TF administration lasts three weeks in cancer patients.[16] Treatment was continued until the earliest event: patient death, appearance of an unresectable disease recurrence, tumor-free survival for 2 years or protocol termination. The 2 year standard treatment period was chosen because of the knowledge that in very high risk Stage I patients and in Stage II patients the likelihood of recurrence approaches 50% during this time.[9]

Interval evaluation of disease status was performed at the Melanoma Clinic every 3 months until the patient had been disease free for 2 years. Study observations were continued semiannually thereafter. These evaluations consisted of history, physical examination, complete blood count, serum chemistry panel, chest radiograph and urinalysis. More frequent examinations or additional testing were performed if warranted.

Any recurrence date was documented as the earliest appearance of a lesion subsequently confirmed to be melanoma. Whenever possible, histologic confirmation of recurrence was obtained. For visceral metastasis, recurrence was considered present when, in the opinion of the investigators, lesions were almost certainly melanoma, *e.g.*, when radiographic studies demonstrated new pulmonary, hepatic, or brain metastasis.

Statistical Analysis

Times to first disease recurrence, Stage III dissemination, and death were analyzed using the Kaplan–Meier life table method.[17] The statistical significance of life table endpoint differences between TF and placebo groups was determined using both the Gehan[18] and log-rank (Mantel)[19] tests. Specified *P*-values are two tailed and are considered significant for $P \leq 0.05$.

Study Medication Preparation

Transfer factor was prepared using a modification of previously described methods.[20] In brief, normal healthy volunteers were selected as donors. Donor leukocytes were harvested by leukophoresis using an Aminco Celltrifuge and were further separated from remaining plasma by centrifugation. The cells were counted, washed, lysed by repeated freezing and thawing, lyophylized, and dialyzed. The dialysate was lyophylized and then suspended in enough sterile, distilled water to obtain a final concentration of 1.67×10^8 lymphocyte cell equivalents per milliliter or 5×10^8 lymphocyte equivalents per 3 milliliter dose. This dose was selected based on previous knowledge of its success in the transfer of cellular immunity to patients with pulmonary melanoma metastases.[14] Placebo consisted of carmelized glucose having a similar appearance and irritative quality as TF. Both TF and placebo were stored at −70° in coded, 3 milliliter, single-use vials until used.

TABLE 1. Distribution of Patient Characteristics

Characteristic	No. of patients in group	
	TF	Placebo
Group total	85	83
Sex		
Male	53	51
Female	32	32
Age (yr)		
<20	2	0
20–39	26	34
40–60	37	30
>60	20	19
Disease stage		
I	35	34
II	50	49
Stratification class		
1	9	10
2	19	22
3	20	22
4	29	25
5	8	4

TF: transfer factor.

Results

One hundred and eighty patients were randomized to the study; of these, 89 were entered into the TF arm and 91 into the placebo arm. Twelve patients were subsequently excluded from the analysis; five were realized to have had active disease at time of entry (one patient, TF; four patients, placebo), one patient did not meet histologic criteria (placebo) and six candidates either never started treatment or had a therapy trial of less than 3 months (three patients, TF; three patients, placebo). Exclusion of these patients was performed before knowledge of their treatment regimen was available to the investigators. A total of 168 evaluable patients remained (85 patients, TF; 83 patients, placebo).

Comparisons of the treatment and control groups revealed similar characteristics in terms of patient sex, age, stage, and stratification (Table 1). Important pathologic prognostic indicators also proved comparable (Table 2).

TABLE 2. Distribution of Pathologic Characteristics

Characteristic	No. of patients in group TF	Placebo
Group total	85	83
Primary lesion Clark's level		
II	1*	0
III	17	15
IV	29	37
V	12	10
Mucosal	2	0
Acral-lentigenous	4	4
No known primary	5	4
Unclassified	14	12
Unknown	1	1
Primary lesion depth		
<1.25	4*	6*
1.25–2.99	33	38
3.00–4.99	16	15
>5.00	17	10
Mucosal	2	0
Acral-lentigenous	4	4
No known primary	5	4
Unclassified	2	5
Unknown	2	1
Primary lesion location		
Extremity	30	25
Trunk	29	40
Head, neck	15	10
Mucosal	2	0
Acral-lentigenous	4	4
No known primary	5	4
Node dissection		
Not done	16	17
Done		
Positive	49	45
Negative	20	21

TF: transfer factor.
* Patients entered trial after resection of nodal metasases (stratification Group 4).

The median follow-up period for all patients was 24.8 months (TF: 22.3 months, placebo: 26.5 months) with a range of 0.5 to 59.3 months (TF: 6.0 to 49.3 months, placebo: 0.5 to 55.3 months). Follow-up for all patients was complete.

Three endpoints were selected for evaluation: time to first recurrence, time to first Stage III disease, and survival. Time to first Stage III disease was included in the analysis because progression to disseminated melanoma provides an early indication of fatal outcome. A therapy capable of slowing the onset of Stage III melanoma, even if it did not have an effect on local-regional recurrence, might favorably influence survival.

The differences in disease-free intervals between treatment and placebo groups approached statistical significance (Fig. 1A); patients receiving TF tended to experience first recurrences more rapidly than controls (P = 0.1004, Gehan; P = 0.0534, logrank). Times to first recurrence were comparable for the Stage I groups (P = 0.5005, Gehan; P = 0.3878, logrank) (Fig. 1B). The adverse trend appeared most pronounced among the Stage II patients (P = 0.1541, Gehan; P = 0.0673, logrank) (Fig. 1C).

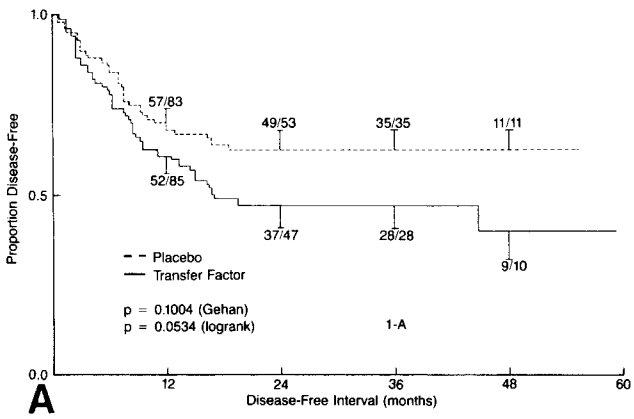
Time to first Stage III disease (Fig. 2A) was significantly shorter for TF-treated participants than for those receiving placebo (P = 0.0164, Gehan; P = 0.0158, logrank). No significant differences were noted when only Stage I patients, treatment and control, were compared (P = 0.2146, Gehan; P = 0.4225, logrank) (Fig. 2B), the Stage II patients contributing most prominently to the disparity between the groups (P = 0.0531, Gehan; P = 0.0184, logrank) (Fig. 2C).

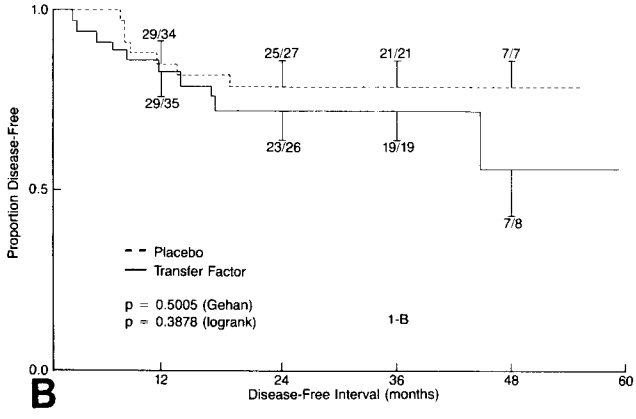
Significant survival differences between TF and placebo groups were not observed, no matter whether comparison was made among all patients (P = 0.1882, Gehan; P = 0.1218, logrank) (Fig. 3A), Stage I subjects (P = 0.0988, Gehan; P = 0.2397, logrank) (Fig. 3B), or participants with Stage II disease (P = 0.4997, Gehan; P = 0.2374, logrank) (Fig. 3C). A more favorable outcome in the placebo-treated group was still suggested by the trend.

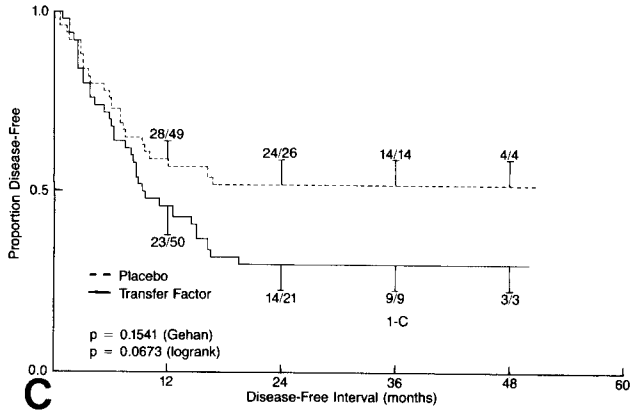
When analyses were performed incorporating survival and recurrence data from the 12 excluded patients, no material impact on the study results could be found. No side effects of TF were observed except for occasional injection site tenderness. One placebo-treated patient and two patients given TF died with no evidence of melanoma.

Discussion

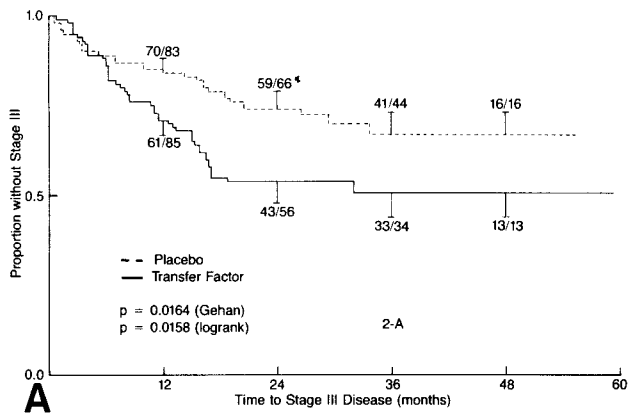
Historically controlled therapy trials often suffer from inadvertant exaggeration of poor outcome in the control group and favorable biases in the selection of the treatment group.[21] Seemingly effective therapies, when evaluated in a randomized fashion, frequently provide less encouraging results. The potential benefit and minimal toxicity of TF observed in early open studies[13–15] indicated the need for this larger randomized, controlled double-blind trial to thoroughly assess TF efficacy. However, like previous well designed evaluations of bacillus calmette guerin vaccine (BCG)[22–24] and levamisole[25] as adjuvant immunotherapy, this study proved TF to be disappointing in the prevention of melanoma recurrences. These most recent results in fact demonstrate favorable trends in the placebo-treated patients as compared to those receiving transfer factor when patients were evaluated for time to first recurrence, time to first Stage III disease, and survival. The difference with regard to one endpoint, time to first Stage III disease, was statistically significant with a more rapid disease progression rate noted in the treatment group, particularly among patients receiving TF after surgical resection of nodal metastases. The increased recurrence rate in the

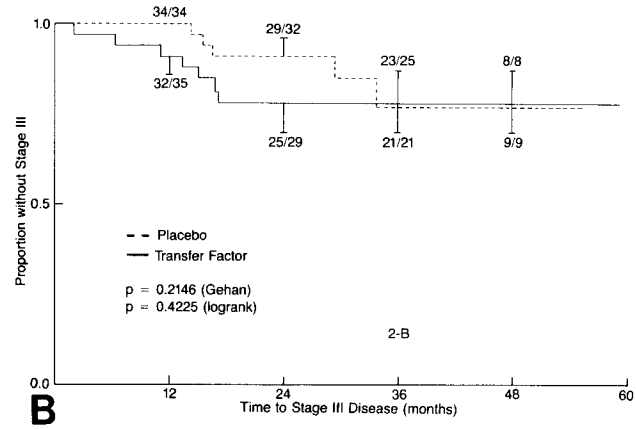


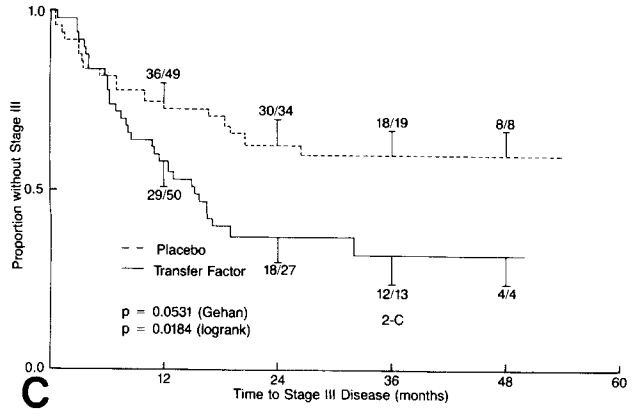




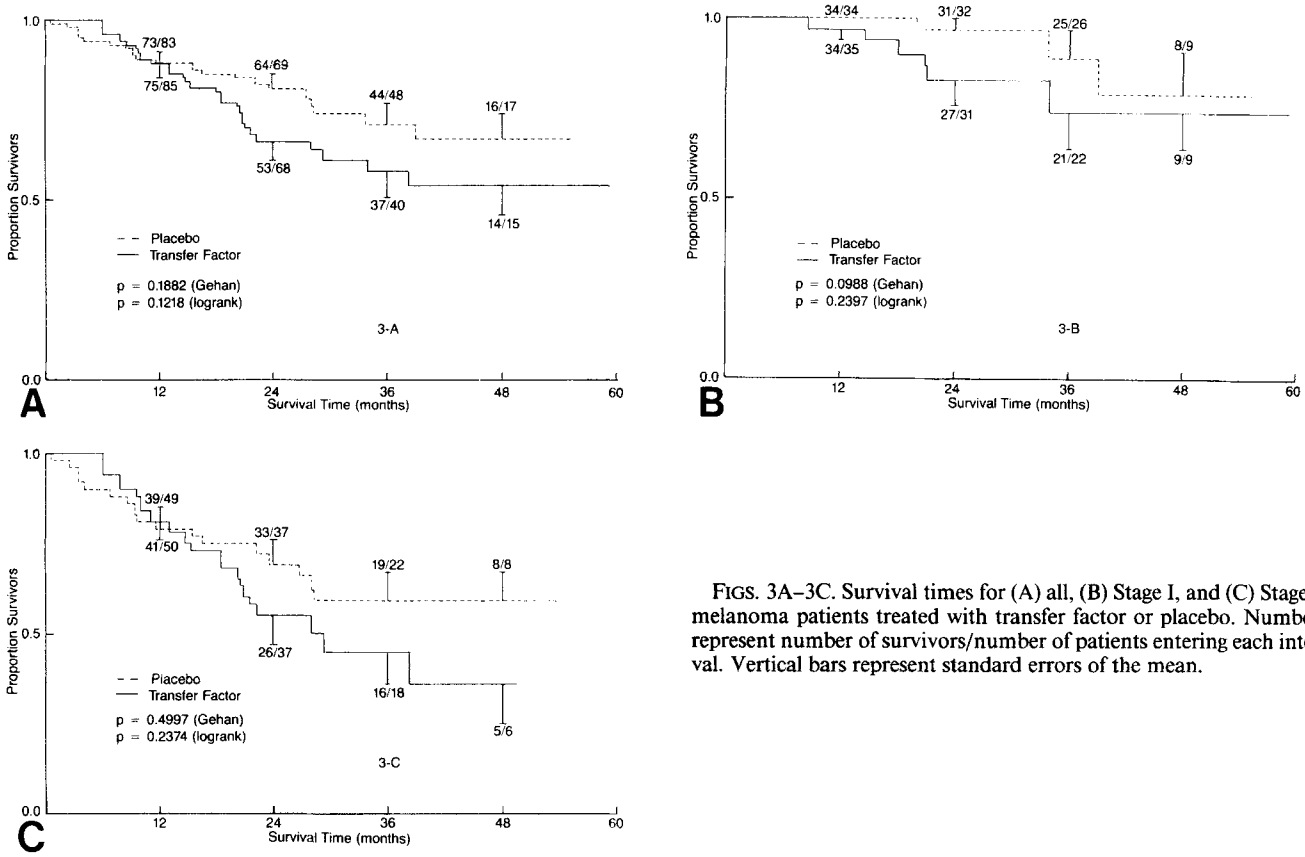
FIGS. 1A–1C. Disease-free intervals for (A) all, (B) Stage I, and (C) Stage II melanoma patients treated with transfer factor or placebo. Numbers represent number of patients disease-free during interval/number of patients entering each interval. Vertical bars represent standard errors of the mean.







FIGS. 2A–2C. Times to progression to Stage III disease for (A) all, (B) Stage I, and (C) Stage II melanoma patients treated with transfer factor or placebo. Numbers represent number of patients without Stage III disease during interval/number of patients entering each interval. Vertical bars represent standard errors of the mean.



FIGS. 3A–3C. Survival times for (A) all, (B) Stage I, and (C) Stage II melanoma patients treated with transfer factor or placebo. Numbers represent number of survivors/number of patients entering each interval. Vertical bars represent standard errors of the mean.

treatment cohort had not translated into significantly increased mortality at the time of data analysis, but an unfavorable trend was apparent.

The study observations extend the tentative conclusions drawn from an earlier Cleveland Clinic randomized TF therapy protocol[26] under which 36 melanoma patients with regional skin and/or node metastases were assigned to receive either surgery followed by TF or surgery alone. An improved median survival time of 40.6 months in the TF patients contrasted with 27 month median control group survival but statistical significance was not shown ($P = 0.17$). Small study size, prognostic differences between patient cohorts, the use of both unselected and patient-related donors, and the unblinded nature of this trial cast uncertainty upon the findings, but TF benefit seemed unlikely.

Randomized trials evaluating adjuvant TF therapy in other types of malignancy have provided mixed results. Patients with nasopharyngeal carcinoma given TF after radiotherapy for local tumor extension showed an unfavorable mortality trend, but statistical significance was not shown.[27] Adjuvant treatment of osteosarcoma,[28,29] mycosis fungoides,[30] and recurrent, low-grade bladder tumors[31] have also demonstrated the inefficacy of TF for these disorders. By contrast, TF benefit has been claimed in the treatment of invasive cervical carcinoma[32] and, in two separate trials,[33,34] an advantage was suggested to its use after primary lung cancer therapy. The reasons for these disparate findings remain elusive. The use of selected TF donors (household contact, cured patients) has not convincingly influenced outcome; TF from specified donors has given both positive[32,33] and negative[27,28] results in randomized trials. Differences in TF preparation, doses and dosing schedules, the method of primary tumor control, and the simultaneous use of chemotherapy and TF also complicate analysis of the varying results.

Certainly, it is clear, based on our findings, that TF is ineffective as surgical adjuvant therapy for melanoma. However, the adverse trends noted in the treatment group are more difficult to interpret. It is conceivable that TF could produce untoward results. Relatively recently the presence of immunologic suppressor substances in TF dialysates has been reported.[35] Such suppressors have been claimed to have potentially detrimental clinical effects when administered as therapy for isolated immune defects in patients with certain chronic infections,[36] but their impact on the course of melanoma, or cancer in general, remains unknown. Because random variation, rather than a true TF effect, remains an alternate explanation for the negative trends, further patient entry and longer follow-up would have been nec-

essary to determine whether or not TF really influences the outcome adversely in patients with melanoma; presumably a true TF effect would have persisted and would have induced a statistically significant survival difference whereas adventitious findings would have been negated by randomization of larger numbers of patients to both study arms. However, the obvious lack of TF benefit demonstrated by the results made continuation of the trial in order to resolve this question unjustifiable.

In summary we conclude that TF has no role in the management of malignant melanoma and that efforts to develop beneficial treatment for this disorder should pursue other approaches.

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