ACTINOMYCIN-D, LEVAMISOLE CHEMOIMMUNOTHERAPY OF REFRACTORY MALIGNANT MELANOMA

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Sixty adult patients with disseminated melanoma refractory to DTIC or Dacarbazine were given chemoimmunotherapy with intermittent high single dose Actinomycin-D and Levamisole. Actinomycin-D was given at a dose of 1.5–2.0 mg/m^2 intravenously every 3 to 4 weeks. Levamisole was given in a dose of 150 mg/day for two consecutive days each week (50 patients) and in a dose of 200 mg every other day (10 patients). Antitumor responses consisted of 2% complete remissions (CR), 2% partial remissions (PR), and 33% disease improvement less than PR or stabilization (S). Comparison of these patients who received Actinomycin-D + Levamisole with those on an immediately preceding study in a similar population where Actinomycin-D was given as a single agent revealed no difference in response rates. Patients who responded to Actinomycin-D + Levamisole (CR + PR + S) survived significantly longer (35 weeks) than nonresponders (12 weeks, $p < 0.01$). Survival was not longer ($p < .05$) in responding patients (CR + PR + S) receiving Actinomycin-D + Levamisole (35 weeks) compared to those responding to Actinomycin-D alone (18 weeks, $p = 0.09$). Hematologic toxicity was tolerable with median lowest granulocyte counts of $1.6 \times 10^3/\mu l$ and platelet counts of 134,000/μl. Other toxic effects were predominantly nausea, vomiting, and mucositis. In those patients who received alternate day Levamisole there was greater gastrointestinal upset as well as fever, rash and central nervous system toxicity which was unacceptable.

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DIMETHYL TRIAZENO IMIDAZOLE CARBOXAMIDE (DTIC; Dacarbazine) is the most effective single chemotherapeutic agent for disseminated melanoma with an overall remission rate of about 20% and complete remission (CR) rate of approximately 5% as determined in several large studies.[1,3] The addition of immunotherapy with Bacillus Calmette-Guerin (BCG) has increased remission duration and survival in those patients responding to DTIC therapy.[7] Patients failing to respond to DTIC treatment have a dismal prognosis. These patients have a median survival of 2–4 months and a very low response rate to subsequent second line drug therapy.[1,5] We recently reported the results of a study in 22 evaluable patients with refractory melanoma employing intermittent high single dose Actinomycin-D.[2] There was an overall response rate of 9% (CR + PR) with an additional 27% of the patients obtaining stabilization of previously progressive disease. These data along with those from other studies[1,11] suggested to us that Actinomycin-D has activity in metastatic melanoma. In an attempt to

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TABLE 1. Clinical and Treatment Characteristics

	Actinomycin-D	Actinomycin-D + Levamisole
Number of patients	23	55
males	14 (61)*	26 (47)
females	9 (39)	39 (53)
Age (yrs.)	42 (28–68)	48 (22–75)
Response data		
% Complete remission	4	2
% Partial remission	4	2
% Stable disease	35	33
% Progressive disease	59	63

* Numbers in parentheses are percentages of patients except for age where the range in years is in parenthesis.

confirm this activity in a larger number of patients and with hope of increasing remission rate, duration and survival, we added the immunorestorative agent Levamisole to the treatment regimen. Levamisole was selected because it had shown promise as an immunopotentiating agent *in vitro* and *in vivo*.[8] There was also clinical evidence of prolongation of disease free interval in patients with breast[12] and bronchogenic carcinoma[13] treated with Levamisole as adjuvant therapy after radiation or surgical treatment of the primary tumor.

MATERIALS AND METHODS

Sixty adult patients with disseminated melanoma were entered on the study. All patients had progressive disease refractory to prior chemotherapy or chemoimmunotherapy regimens which included DTIC. Patients were evaluated before therapy with tumor measurements, blood counts and sera chemistries. Blood counts were repeated 1–2 times a week with chemistries and tumor measurements repeated before every course of therapy. Informed consent was obtained from all patients before therapy according to institutional policy. The starting dose of Actinomycin-D in most patients was 2.0 mg/m^2 with some receiving 1.5 mg/m^2 because of compromised bone marrow reserve. The drug was given intravenously in 100–250 cc of 5% dextrose and water, repeated at 4 week intervals with the dose adjusted depending on myelosuppressive and gastrointestinal toxicity. Levamisole was given at a dose of 50 mg orally 3 times a day on 2 consecutive days each week (days 7, 8, 15, 16) in 50 patients (weekly Levamisole). Ten patients received intensive Levamisole as part of a Phase I study of this compound. The dosage was 200 mg orally every other day with the dose subsequently reduced for excessive toxicity.

Complete remission (CR) was defined as disappearance of all clinical evidence of active tumor and tumor related symptoms for a minimum of 4 weeks. Partial remission (PR) was defined as a ≥50% decrease in the sum of the products of the longest perpendicular diameters of all measurable lesions lasting at least 4 weeks without the appearance of new lesions. Stable disease (S) consisted of a steady state of response less than PR for at least 4 weeks or no evidence of progressive disease for at least 8 weeks. Progressive disease was defined as an increase of any lesion by ≥25% or the appearance of any new lesions. Survival was computed and plotted according to the method of Kaplan and Meier[9] and statistically compared by the Gehan modification of the generalized Wilcoxon test.[6]

RESULTS

Response

Patients evaluable for response received one full course of chemoimmunotherapy and were alive and evaluable for tumor response 4 weeks later. Fifty-five patients met these criteria (Table 1). The five patients not evaluable for response included four early deaths and one patient with inadequate follow-up data to assess tumor behavior. The overall response rate (CR + PR + S) was 36%. There was one CR (2%) in a male patient with soft tissue and nodal disease who remains in remission and on therapy at 20 months. One PR (2%) was seen of short duration (4 weeks) in a patient also with soft tissue disease. There were 18 patients (33%) with stable disease for a median of 4.5 months (range 3 to 9 months). Three of these 18 patients had definite tumor regression less than PR; two of these involved soft tissue or nodal disease and one liver and lung metastatic deposits. Therefore 5 (9%) of the evaluable patients had clinical evidence of tumor regression. Four patients are alive, two remain on Actinomycin-D + Levamisole (1 CR, 1 Stable) and two are receiving different chemoimmunotherapy, because of tumor progression. Thirty-five patients (63%) had definite evidence of progressive disease. In 19 (54%) it occurred after one course of therapy and in 16 (46%) after two courses.

Survival

Survival data were obtained for 55 evaluable patients who received Actinomycin-D + Levamisole and for 23 patients with metastatic melanoma who received Actinomycin-D alone at comparable doses in the immediately preceding study. Early deaths (within 2 weeks from treatment onset) were excluded in both patient groups. Clinical characteristics and response data for both groups are shown in Table 1.

Patients responding (CR + PR + S) to Actinomycin-D + Levamisole therapy had a statistically significant longer survival than those who did not. The patients who achieved a CR or PR with Actinomycin-D alone had a longer survival than patients with progressive disease however when the stable patients were included with the responders this was not true.

The Actinomycin-D + Levamisole responders (CR + PR + S) had a median survival of 35 weeks compared with 12 weeks for the nonresponders ($p = 0.01$) (Fig. 1). In an attempt to determine the contribution of Levamisole, we compared the survival of responders (CR + PR + S) to Actinomycin-D + Levamisole to responders (CR + PR + S) to Actinomycin-D. The median survival for the responders to Actinomycin-D + Levamisole was longer (35 weeks versus 18 weeks) than that for the Actinomycin-D alone responders (Fig. 2). Although this was not statistically significant at the 5% level, it was at $p = 0.09$ which suggests a trend but does not demonstrate a survival advantage for the Actinomycin-D + Levamisole responders.

Toxicity

Hematologic toxicity was evaluable in 34 courses of therapy in patients receiving weekly Levamisole. Twenty-five of the courses were at the 2.0 mg/m² dose of Actinomycin-D and 9 at 1.5 mg/m². The lowest recorded points of myelosuppression on this regimen are shown in Table 2 and compared with those on seven courses of intensive Levamisole with 2.0 mg/m² Actinomycin-D. The duration of granulocytopenia and thrombocytopenia was similar in both groups and similar to that in our previous study of Actinomycin-D. No cumulative myelosuppression was seen. Nine patients started at 2.0 mg/m² Actinomycin-D required a dose reduction to 1.5 mg/m² and 3 were escalated to 2.5 mg/m² based upon drug effects. Five patients started at 1.5 mg/m² were subsequently escalated to 1.75 or 2.0 mg/m².

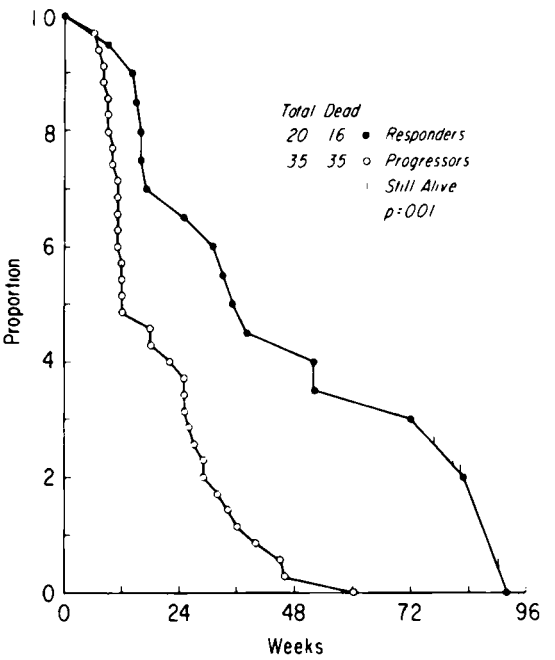

FIG. 1. Comparison of survival for patients responding and progressing on Actinomycin-D + Levamisole chemotherapy.

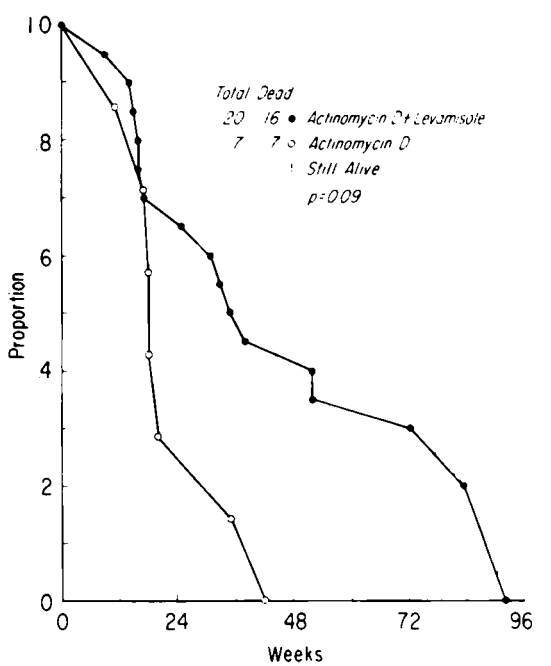

FIG. 2. Comparison of survival for patients responding to Actinomycin-D + Levamisole versus those responding to Actinomycin-D.

TABLE 2. Hematologic Toxicity*

Initial course at actinomycin dose	No. courses evaluable	Lowest granulocyte count × 10^3 μl	Day lowest	Lowest platelet count × 10^3 μl	Day lowest
Actinomycin-D + Weekly Levamisole					
2.0 mg/m²	25	1.6 (0–9.8)	15 (5–28)	134 (20–511)	9 (7–27)
1.5 mg/m²	9	1.5 (.4–2.4)	17 (7–36)	131 (18–99)	10 (7–27)
Actinomycin-D + Intensive daily Levamisole					
2.0 mg/m²	7	1.4 (0–3.3)	12 (9–25)	110 (20–176)	8 (6–9)

* Lowest recorded granulocyte counts and day of their observation are given as median values with range in parentheses.

Complications related to myelosuppression were seen with the combination of Actinomycin-D and Levamisole. There were six episodes of fever unrelated to drug administration, which all occurred in patients with fewer than 500 absolute granulocytes/μl. There were no documented infections and all patients with fever responded without difficulty to antibiotics or granulocyte recovery. There were 3 episodes of bleeding encountered in the patients, all of whom had platelet counts greater than 143,000/μl. One patient on an oral anticoagulant had hematuria, another an upper gastrointestinal hemorrhage, the source of which was not determined but which did respond to conservative treatment, and the third a mild episode of epistaxis. None of these events could be attributed to therapy. Five percent of evaluable courses of Actinomycin-D + weekly Levamisole had platelet counts fewer than 50,000/μl and 17% below 100,000. Fourteen percent of evaluable courses of intensive Levamisole + Actinomycin-D had platelet counts fewer than 50,000/μl and 42% fewer than 100,000/μl. Comparison of absolute granulocyte levels showed 14% of the courses of Actinomycin-D + weekly Levamisole had counts fewer than 1000/μl while 43% of the courses of intensive Levamisole + Actinomycin-D had granulocyte counts below this level. Only 4 patients (5%) required hospitalization for fever or bleeding. Actinomycin-D was discontinued in 2 patients because of unacceptable gastrointestinal toxicity. There were no drug-related deaths.

TABLE 3. Other Toxicity

	Actinomycin-D + weekly Levamisole	Actinomycin-D + intensive daily Levamisole	All courses Actinomycin-D + Levamisole
No. of patients evaluable	40	10	50
No. of courses evaluable	77	28	105
Mucositis*	12	20	10
Nausea, vomiting* 3 days	17	40	14
Rash*	5	30	6
Fever*	2	50	9
Bleeding*	7	0	4
Diarrhea*	5	0	3
Central nervous system abnormalities*	0	10	1
Hospitalization*	2	30	4

* All numbers in percentages of patients with specific toxicity except last column which is percentage of courses with specific toxicity.

Other nonhematologic toxicity was evaluable in 50 patients. Forty of these received weekly Levamisole and 10 received intensive alternate day Levamisole. Table 3 shows the incidence of other toxicities in both patient groups. In most instances, toxicity was more frequent and severe with the intensive Levamisole than with the weekly Levamisole. High drug-induced fever, nausea, vomiting and skin rash were the most common side-effects from the intensive Levamisole regimen and could be attributed to the Levamisole therapy. Although some of these side-effects were seen in patients given the Actinomycin-D alone, the incidence was much higher in patients given the Actinomycin-D with Levamisole and particularly the intensive Levamisole regimen. One patient of note developed headache and

seizures culminating in coma, from which he recovered, after receiving the intensive Levamisole regimen with Actinomycin-D. Nausea, vomiting, and mucositis were seen in patients receiving Actinomycin-D with weekly Levamisole; however, the frequency of these side-effects did not differ substantially from those in our previous study with Actinomycin-D alone. Weekly Levamisole did not appear to give additive toxicity to Actinomycin-D but with intensive Levamisole therapy there was a substantial, unacceptable increase in toxicity and morbidity.

DISCUSSION

This study of Actinomycin-D + Levamisole in DTIC refractory patients with disseminated melanoma when compared with our previous study in a similar population gives a CR rate of 3%, a PR rate of 3% and a 29% rate of disease stabilization in 78 evaluable patients. The addition of Levamisole to Actinomycin-D did not significantly increase remission rate or median duration of remission (4 months vs. 2 months). The 35% disease stability rate in DTIC refractory patients with acceptable toxicity suggests the use of intermittent single dose Actinomycin-D in patients previously untreated or with an earlier stage of melanoma; perhaps in conjunction with DTIC or other chemoimmunotherapy regimens.

The toxicity of Actinomycin-D with or without weekly Levamisole is tolerable without major morbidity and with myelosuppression, nausea, vomiting and mucositis the major manifestations. Intensive alternate day Levamisole as given in this study had major unacceptable morbidity manifested by excessive gastrointestinal and skin toxicity which was additive or synergistic with that from Actinomycin-D. The occurrence of fever and central nervous system abnormalities seen in this study was severe and related to intensive high-dose alternate-day Levamisole therapy.

The survival data showed that patients responding to Actinomycin-D + Levamisole live longer than patients with progressive disease. The significant prolongation of median survival in the responders (CR + PR + S) of 35 weeks is similar to survival in previously untreated patients responding (CR + PR + S) to DTIC alone (6–11 months, median 9 months) (4, 7, 10). The survival of patients with progressive disease was dismal (median

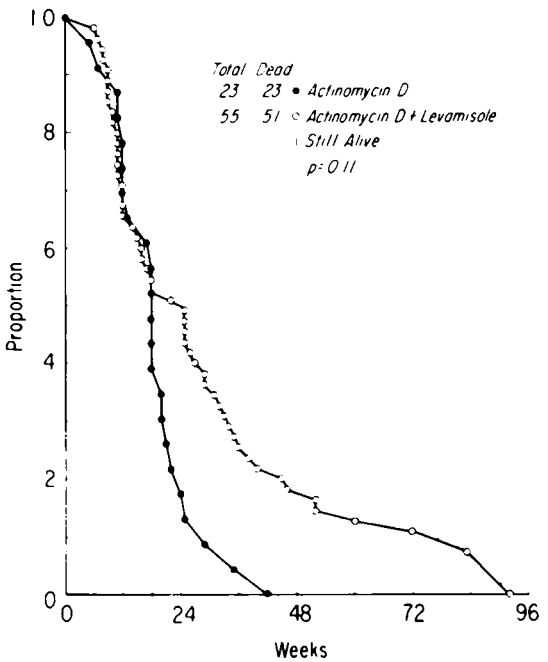

FIG. 3. Comparison of survival for all patients treated with Actinomycin-D + Levamisole versus all patients treated with Actinomycin-D.

12.0 weeks) and similar to that reported for patients failing first line therapy with DTIC.[9]

The survival for all patients on Actinomycin-D + Levamisole was not significantly longer than that for Actinomycin-D (Fig. 3). The median survivals were 24 weeks for Actinomycin-D + Levamisole and 18 weeks for Actinomycin-D ($p = 0.11$). The 25th percentile survival was 37 weeks for the Levamisole group compared with 21 weeks for the Actinomycin-D alone group.

Although the survival difference between patients responding (CR + PR + S) to Actinomycin-D + Levamisole and those responding (CR + PR + S) to Actinomycin-D alone (35 weeks versus 18 weeks) is not statistically significant ($p = 0.09$), 4 of the 20 responding patients on the Actinomycin-D + Levamisole study are alive at 77+ weeks while no patient on the Actinomycin-D alone study survived longer than 42 weeks.

Our data suggest minimal antitumor activity of single dose Actinomycin-D in previously treated patients with melanoma. The addition of Levamisole to Actinomycin-D did not increase the remission rate or demonstrate a significant survival advantage for patients responding to the drug combination. Further investigations of Levamisole in combination

with DTIC in previously untreated patients or patients with earlier disease are needed to determine the effect of this agent in patients with malignant melanoma.

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