LOW-DOSE CYCLOPHOSPHAMIDE AND LOW-DOSE INTERLEUKIN-2 FOR MALIGNANT MELANOMA*

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MALIGNANT melanoma is a tumor whose incidence is increasing steadily, probably because of intense intermittent sun exposure among individuals with indoor occupations. Nearly 28,000 new cases will occur during 1988 in the United States.[1] Melanoma is therefore not a rare tumor, and with better awareness of its early signs the primary lesion is becoming more commonly recognized by internists, dentists, and ophthalmologists, as well as by the patients themselves. While surgery of primary melanoma can be curative in the early stages, the treatment of advanced disease is extremely disappointing. Neither chemotherapy nor radiation therapy is particularly helpful, although there have been continual reports of successful intensive chemotherapy regimens.

The report by Rosenberg and colleagues in 1985[2] of the usefulness of adoptive immunotherapy with lymphokine activated killer cells given together with interleukin-2, in the treatment of melanoma was met with appropriate enthusiasm. Interleukin-2 was reported by Rosenberg and colleagues

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to elicit lymphokine activated killer cells from the leukocytes of patients with melanoma when the two were incubated for four days in vitro. These cells, upon reinfusion into the patient together with varying doses of additional interleukin-2 intravenously, were effective in causing five of ten patients with melanoma to have significant regressions of their disease.[2] The strategy of ex vivo activation was necessitated by that group's findings that interleukin-2 given by itself was incapable of activating lymphokine activated killer cells sufficiently to cause clinical responses.[3] Most recently, however, the same group has reported preliminary success with high doses of interleukin-2 alone in vivo,[4,5] although with a response rate of only 13% versus the 22% noted with lymphokine activated killer cell reinfusion. Although the regression of such a resistant tumor as melanoma was highly gratifying, the toxicity of the regimens that have been described has been substantial even with modifications.[6] In particular, elderly patients have been at a significant risk of developing interstitial pulmonary edema as part of a diffuse capillary leak syndrome, and four deaths have been attributable to the regimen.[5] The cost and complexity of ex vivo activation of lymphokine activated killer cells are considerable, which militates against broad adoption of the technique even in modern general hospitals. Finally, in vitro incubation of leukocytes with interleukin-2 for several days require extensive tissue culture facilities, and carries a risk of hepatitis.

We have investigated whether interleukin-2 given directly to patients with melanoma could affect the disease. Because interleukin-2 stimulates all subsets of T cells rather than simply the T-helper and T-cytolytic cells that one might want to activate preferentially, we designed a strategy that might decrease T- suppressor cells before interleukin-2 was administered. This might permit proliferation and maturation of other subclasses unimpeded by suppressor influences. A dose of 350mg/m^2 of cyclophosphamide was selected because of the work of others[7,8] as well as our own pilot investigations (Hengst, J.C.D. and Mitchel, M.S., unpublished data) which showed that this drug at doses of 300-500 mg/m^2 selectively depleted suppressor T cells in man. We further designed a schedule of administration of interleukin-2 given to outpatients in a day-hospital setting that permitted close observation for eight hours or more, and which incorporated a week's respite from therapy after each of three two-week cycles of treatment.

PATIENTS

Thirty-eight patients with disseminated malignant melanoma have been studied, unselected except by preestablished entry criteria. These included measurable lesions on physical examination or on roentgenograms or com-

puted tomographic scans of the chest or abdomen, the absence of central nervous system involvement, a Karnofsky performance status of at least 70%, and either resistance to "standard" chemotherapy or refusal by the patient to undergo such chemotherapy after a discussion with the referring physician. Patients ranged from 25 to 75, and eight were older than 50. No one was excluded because of age, except for those younger than 18. Pregnancy and lactation were also criteria for ineligibility, as were psychological or social impediments to compliance. The diagnosis was always confirmed by our pathologists after review of the original slides. A written consent form was signed by each patient after a full explanation of the study was given by the physician. All patients reported here were treated with at least one full cycle of cyclophosphamide plus interleukin-2, although the critical evaluation of a response to the treatment was performed after three complete cycles (one full course).

Twenty four patients were male and 14 were female. Sites of disease included subcutaneous and intradermal skin nodules (21 patients), pulmonary metastases (16 patients), lymph nodes (14 patients), liver metastases (10 patients), bone lesions (six patients), pelvic mass (four patients), adrenal metastases (four patients), chest wall mass (one patient), splenic metastasis (one patient), and a renal metastasis (one patient). Most (26 patients) were untreated with chemotherapy. Twenty two of the 38 had received some form of immunotherapy, including 15 who were treated with lysate of melanoma cells and the immunological adjuvant DETOX (detoxified endotoxin and mycobacterial cell wall skeletons[9] in a study to be reported separately.[14] Four patients had had no therapy other than surgery. No therapy of any kind was permitted within the three weeks prior to the start of the cyclophosphamide plus interleukin-2 regimen, during which the patient had recovered from any side effects of previous treatment, and the disease was either stable or progressing.

REGIMEN

All treatment was administered in a day-hospital outpatient setting, where prolonged observation was possible without admitting the patient to a ward. Cyclophosphamide was given as an intravenous bolus of 350 mg/m^2 three days before the start of the interleukin-2 treatment on a Friday morning. Beginning three days later, on a Monday morning, interleukin-2 was given as a 15-minute intravenous infusion, and was then repeated at the same level on the second through fifth and eighth through 12th days, doses being omitted

on Saturday and Sunday. The dose of interleukin-2 during the first two-week "cycle" was 3.6 million units/m^2/d. Each patient was observed eight hours on the first day. By the fifth day the patient was permitted to go home within one to two hours if he had no serious toxicity, such as hypotension or uncontrollable fever. We found that pretreatment with acetaminophen, 650 mg, repeated every four hours as needed, with indomethacin 25 mg if needed, limited the severity of the fever although it did not entirely prevent it. Meperidine, 25 to 50 mg intravenously given once or twice p.r.n., was helpful in controlling the shaking chills that preceded the fever. Indomethacin, 25 mg, was also given every four hours if needed for severe arthritis or fever unless gastrointestinal toxicity became intolerable. Medicines specifically excluded were corticosteroids and type two antihistamines.

The two-week cycle of cyclophosphamide plus interleukin-2 was repeated at least twice more to complete a three-cycle course of therapy. 3.6 million units/m^2 were most often used through all three cycles. The attending physician could, at his discretion, increase the dose to 5.4 million units/m^2 for the 2nd and 7.2 million units/m^2 for the third, if the patient had tolerated earlier treatments without severe toxicity as explicitly defined by World Health Organization criteria, modified by Cetus. However, after the first 20 patients, we rarely increased the dose above the starting level. If patients had at least a minor response (see definitions in the next section) after three two-week cycles, they were eligible to continue the treatment. Stable disease or progression after a full course of three cycles mandated discontinuation of therapy. After a complete remission, two or three more cycles of treatment were given, after which treatment was stopped and the unmaintained response was followed. If patients had sustained a partial remission after three cycles they were treated with at least three more cycles or until either a complete remission or progression occurred. Maintenance therapy, of one cycle of treatment every six weeks, was given those who had a stable partial remission after more intensive treatment. In one instance, a patient with a partial response of lymph node lesions but a minor response of subcutaneous lesions, was kept on this maintenance regimen, and in fact has continued on maintenance beyond 18 months.

Except for the earliest patients, most were given at least a two-week interval after the first three cycles to provide respite from the stresses of continual treatment. The oldest patients on study and those treated for many months were often permitted a four-week interval after each full course of three cycles.

Criteria of Response

Standard criteria were used to judge response to treatment. Thus, complete remission was defined as the complete disappearance of all measured lesions for at least four weeks and failure of new lesions to appear during that time. Partial remission was defined as 50% decrease in the sum of products of the greatest perpendicular dimensions of all measured lesions, lasting at least four weeks, and without the appearance of new lesions. A minor response was at least a 25% but less than a 50% diminution in the sum of the products of the perpendicular diameters of all measured lesions or a 50% decrease in that sum that lasted less than four weeks. A mixed response was >50% shrinkage at one or more sites, e.g., the skin or lymph nodes, but with no change or progression at another, e.g., pelvic mass. Stable disease meant no change or a diminution of less than 25% in the sum of the products of diameters of measured lesions. Progression was greater than 25% increase in the sum of the products of diameters of all measured lesions. "No response" to therapy, as used here, comprises both stable and progressive disease.

Immunological Studies

We monitored the immunological effects of our regimen in several different ways. Natural killer cells and lymphokine activated killer cells were measured twice before and on the last day of each two-week cycle. Off-study immunological measurements were also made 72 hours after completion of the final two-week cycle. Standard assays for these cells were used[10-12] except that the cells we have termed lymphokine activated killer cells were tested against cultured melanoma cells resistant to natural killer cells rather than against fresh tumor cells as described in the original paper by Grimm et al.[10] With this proviso, we shall refer to the cells as lymphokine activated killer cells. Besides melanoma, other natural killer resistant tumor cells, such as Raji and Daudi B-cell lymphomas and squamous lung carcinoma cells, as well as allogeneic lymphoblasts, were also tested in randomly chosen patients. The tumor cells, but not the lymphoblasts, were found to be lysed by cytolytic mononuclear cells, supporting their close similarity, if not identity, with bona fide lymphokine activated killer cells. Various ratios of effector to target cell were tested, ranging from 200:1 to 25:1 for lymphokine activated killer cell assays and from 40:1 to 2.5:1 for natural killer cell assays. We calculated the number of "lytic units" of each type of killer cell by best-fit regression analysis, comparing linear, logarithmic, exponential and power curves, with a computer program ("Curvefit") created by Mr. Jeffrey S. Mitchell of New Haven, CT. The logarithmic and power curves proved most

TABLE I. RESULTS OF TREATMENT

Complete remissions	2	(5.3%)		
			23.7%	
Partial remissions	7	(18.4%)		
				26.3%
Long-term minor response	1	(2.6%)		
Minor responses	4	(10.5%)		
Mixed responses	4	(10.5%)		
Progression	20	(52.6%)		
Total	38	(100%)		

useful in this regard. The results are expressed in "lytic units" per 10 million effector cells, where one lytic unit is number of cells that can kill 30% of the target cells in four hours. Fisher's two-tailed exact test was used to evaluate the statistical significance of differences between groups.

Serial measurements of concanavalin A-inducible suppressor T cells were made in 17 patients[13] immediately before cyclophosphamide and then three and six days later (i.e., three days before interleukin-2 and then before the first and fourth doses).

RESULTS

Therapeutic efficacy. Nine of the 38 patients (23.7%) sustained partial or complete remission, and one (2.6%) has had a long-term maintained minor remission for more than 18 months (Table I). There were two complete remissions, one in a patient with extensive subcutaneous metastases and one in a patient with three lung nodules and an enlarged hilar lymph node. There were also seven partial remissions. One of the latter included disappearance of all lesions in the liver and skin and two of three lung nodules, with only a residual 1 cm pulmonary nodule. A 10th patient, a 50-year-old man, had a 50% decrease in the size of a large inguinal lymph node, with a lesser degree of decrease of a large number of intradermal and subcutaneous nodules. His disease, which had been inexorably progressive despite several previous types of treatment, has been entirely stable for more than 18 months on maintenance cyclophosphamide plus interleukin-2 treatment. This long-term response has been highly significantly clinically, even though technically a "minor response."

Minor responses were found in a total of 5 patients (13.1%), including this patient, and mixed responses were noted in another four patients (10.5%).

In general, softening or flattening of skin nodules was frequently discernible after the first two-week cycle, but optimal responses usually required six cycles or more.

TABLE II. RESULTS OF TREATMENT BY SITE

Site of disease	*Number of patients*	*Complete remission*	*Partial remission*	*Duration in months*
Liver	10	2	2	CR: 20; 7+ PR: 13; 10
Lung	16	1	3	CR: 8 PR: 20; 3; 2
Subcutaneous	21	2	6	CR: 20; 1.5 PR: 16; 3; 2; 2: 1; 1
Lymph node	14	1	3	CR: 8 PR: 15; 18+; 2
Bone	6	0	0	
Pelvic, renal	5	0	0	
Adrenal	4	0	0	

CR = complete remission, PR = partial remission. Durations of response are given from the date a CR was achieved or the date therapy was begun for a PR, as per convention.

Table II shows the results of treatment by sites of disease. Of most interest to us were the responses of the four of 10 patients with metastases to the liver, a site which in melanoma rarely responds to systemic treatment. There was a complete remission of eight large liver metastases in a 42-year-old woman and in approximately 12 liver metastases, including one of 5 × 4 cm in a 45-year-old man. Both of those patients also had disappearance of all of their three to five small skin nodules. The man had new disease in his bones, which led to discontinuation of his treatment, but has not had reappearance of liver lesions in more than nine months. The woman had two of three lung nodules disappear as well. Although overall she had only a partial remission by strict criteria, with a residual 1 cm lung nodule we elected to discontinue her treatment after one year (15 cycles). She remained in unmaintained remission for eight months, relapsing with brain metastases. There was no recurrence in previously responsive sites outside the central nervous system. Her total dose of interleukin-2 was 540 million units/m^2, the highest cumulative amount administered in our study. Two other patients had >50% shrinkage of liver lesions, which persisted for at least six months. Their responses lasted nearly a year before they too died of brain metastases.

Eight of 21 patients with subcutaneous nodules responded, with two complete and six partial remissions. Two responders had long-term remissions lasting more than 18 months or continual maintenance in one instance and even upon discontinuation of treatment in another. A third patient was nearing a complete remission but had pernicious anorexia and depression, which forced his removal from the study despite his continuing response. There were also minor responses in six patients.

Metastases to the lung were also responsive in four of 16 patients, one of whom had a complete remission in that site. He received two further cycles of treatment, and then was observed without treatment following the protocol. He relapsed with brain metastases after eight months and died shortly thereafter, but his lung nodules never returned. Among the partial responders was the oldest patient on study, a 75-year-old man, who tolerated six cycles of treatment.

Lymph node metastases responded in four of 14 instances, with one complete and three partial responses, and four other minor responses. The complete regression was that of a large hilar node in the patient noted above who had a complete remission of lung metastases. Three of the four responses persisted for eight to more than 18 months, and the fourth response was limited by relapse in the brain which forced temporary discontinuation of treatment.

Unresponsive sites comprised bone, adrenal (see Addendum), renal, and pelvic metastases, where none of 13 patients had a response. In fact, three patients developed or had exacerbations of bone pain that forced discontinuation of therapy that had beneficially affected other sites.

The median duration of complete or partial remissions is nine months, with a mean of >10.1+ months. Four patients have had more than 12 months of remission on continued treatment.

Toxicity. The regimen we have described was generally well tolerated, although accompanied by moderate degrees of toxicity (Table III). Severe side effects were found in 10-15% of four categories: fatigue, fever, nausea/vomiting and chills/rigors, and in 0 to 9% in the remaining nine categories. Only two patients required admission to hospital as a result of the toxicity: both were overnight stays either for repletion of fluids or for fatigue. Hypotension to some degree was found in two thirds of the patients, but was usually mild to moderate. Severe hypotension (Grade III) was found in only four patients (10% of the entire group). Episodes of severe hypotension occurred on one occasion during the treatments of three patients, all of whom then continued on study at reduced levels of interleukin-2. An episode of hypotension also occurred early in the treatment of patient No. 1, who subsequently tolerated a total of 15 cycles, all at 3.6 million units/m^2. Elderly patients had the most difficulty with the regimen, requiring more encouragement to continue than did the others. Only one patient left the study because of a lack of tolerance, but diminution of dose and an increase in the interval between courses of three cycles were necessary for two of the oldest patients.

Shaking chills, fever to a maximum of 103° F (39.4° C), nausea and/or vomiting, and fatigue were nearly universal. Fluid retention, arthralgia/ar-

TABLE III. TOXICITY

Toxic effect	*Number of patients experiencing effect*	*Number with grade III toxicity (% of entire group)*	
Fatigue	40	6	(15%)
Fever	39	4	(10%)
Nausea, vomiting	38	5	(12%)
Chills, rigors	34	3	(7%)
Hypotension	28	4	(10%)
Diarrhea	27	2	(5%)
Allergies, rash	26	1	(2%)
Fluid retention	25	2	(5%)
Dry cough, dyspnea	25	1	(2%)
Arthralgias, arthritis	24	2	(5%)
Myalgia	23	2	(5%)
Catarrhal symptoms	15	0	(0%)
Anemia	12	1	(2%)

Data on 41 patients entered onto the study are shown, including three not evaluable for a clinical response. World Health Organization criteria were used except for Hypotension, which was modified as described in the text, and Fluid Retention (weight gain), which was a category specifically added for IL-2 protocols by Cetus.

thritis, myalgia, diarrhea, dry hacking cough, and rash were also common. Diuresis often ensued spontaneously over the weekend following five days of treatment, and in any event fluid retention always responded to furosemide. Joint symptoms were severe in only one patient, and abated within a few days of cessation of a cycle of treatment. Indomethacin or ibuprofen helped to mitigate this symptom, but did not entirely counteract it. One patient with a previous history of rheumatoid arthritis developed an erythematous rash and a true arthritis with her first course of treatment, but on subsequent treatments only arthralgia without overt swelling or rash was noted. A dry hacking cough, responding to cough suppressants, and respiratory symptoms were also found in two thirds of the group, which suggested an interleukin-2 induced bronchitis. Eosinophilia (>10% of total leukocytes) was noted in 20 patients, and 23 patients complained of myalgia. Many of these symptoms were what one might have expected from a lymphokine active in inflammatory processes.

A mild to moderate decrease in hemoglobin was also found in 12 patients, but no significant changes in leukocytes or platelets were noted. The cyclophosphamide caused minor to moderate nausea lasting one to three days in one half the patients, and frequently caused a flareup in arthralgia. No toxic effects on hair, bone marrow, or bladder were found.

Immunological studies. An increase in lymphokine activated killer cell

TABLE IV. IMMUNOLOGICAL CORRELATES OF CLINICAL RESPONSE

a) Induction of lymphokine activated killer cell activity versus response

	*Response**	*No response*	*Total*
LAK increased†	9	15	24
LAK not increased	0	14	14

*Comprises complete and partial remissions
†>10 lytic units maximal increase during treatment
p=0.014, Fisher's exact test

b) Induction of natural killer cell activity versus response

	*Response**	*No response*	*Total*
NK increased >1,000†	7	18	25
NK increased <1,000†	2	11	13

*Comprises complete and partial remissions
†Maximal increase in lytic units during treatment
p=0.46, Fisher's exact test

activity was significantly correlated with a clinical response (p=0.014) (Table IVa). All nine patients with a partial or complete remission had lymphokine activated killer cell activity increased to a level of 10 lytic units per 10^7 cells or greater. Conversely, none of the 14 patients who failed to develop an increase in lymphokine activated killer activity had a clinical remission. Activity was also increased in 15 nonresponding patients, indicating that the induction of lymphokine activated killer cells was necessary but not sufficient for a remission. However, failure to show an increase in cells portended a failure to respond clinically.

In contrast, as indicated in Table IVb, natural killer cell activity was consistently stimulated in all patients and showed no significant correlation with clinical response (p=0.46). An increase in natural killer cell activity to some degree above baseline occurred not only in the nine responders, but also in all 29 nonresponders. Even considering those patients who had a large increase of more than 1,000 lytic units per 10^7 cells there was no significant correlation. Thus, although seven of nine responders had an increase of more than 1,000 lytic units per 10^7 cells, 18 of the 29 nonresponders also showed such an increase.

No significant preexisting levels of lymphokine activated killer cells were measurable in the circulation. After cyclophosphamide and interleukin-2, those patients who had an increase usually had an incremental increase in the levels of lymphokine activated killer cells with successive cycles, but there was a rapid fall-off within three days after the end of a two-week cycle. This characteristic requirement for the continued presence of interleukin-2 lent

further credence that they are lymphokine activated killer cells rather than another type of cytolytic lymphocyte, such as cytotoxic T cells. However, two patients have had an elevation of cytotoxic T cells too. One was the woman who sustained a complete remission of her liver metastases and the other was the man with a long-term minor response. The former had the highest level of lymphokine activated killer cells in the study, nearly 5,000 lytic units, after her 15th and final cycle of treatment. Cold target competition assays performed during her last cycle of treatment and after the 17th month of treatment of the male patient revealed cytolytic lymphocytes specifically inhibitable by melanoma cells. These cells, which are CD3 positive and can be blocked by antibodies to CD3, coexisted with lymphokine activated killer cells with a much broader reactivity against tumor cells.

Concanavalin-A-inducible suppressor T cells were not consistently reduced by cyclophosphamide in the 17 patients studied. In eight of the patients there was reduction of suppressor T cells three days after the cyclophosphamide, but on day six, three days after interleukin-2 was begun, the suppressor cell activity had returned to its pretreatment level. These results contrasted with previous findings in patients who were given cyclophosphamide alone, where the decrease persisted at least until day six after cyclophosphamide (Hengst, J.C.D. and Mitchell, M.S., unpublished data), and usually until day 28.[9] Unfortunately, we did not have the opportunity to study patients given interleukin-2 alone to determine what levels of suppressor activity would have been elicited by interleukin-2 had cyclophosphamide not been given.

Lack of influence of indomethacin or fever on the clinical response. As shown in Table V, there was no apparent influence either of high fever (temperature elevation to > 101°F) or the administration of the prostaglandin antagonist indomethacin on the clinical response to the regimen. It was conceivable that each of those influences might have acted independently to mediate the effects of the regimen. In particular, antagonism of prostaglandin synthetase might have affected suppressor macrophage function and thus augmented the immunological stimulatory effects of interleukin-2. However, we could not find statistical evidence here that those patients who received indomethacin for high fevers or arthralgias fared differently from those who did not.

DISCUSSION

This investigation has shown that a regimen of low-dose cyclophosphamide and low-dose interleukin-2 was effective in the treatment of

TABLE V. INDOMETHACIN OR FEVER >101F VERSUS RESPONSE

Response	*Indomethacin*	*Fever*
CR	2/2	2/2
PR	5/6	6/6
Minor	4/5	5/5
None	11/17	15/17

Indomethacin: CR + PR vs. Minor + None $p = 0.28$, by Fisher's exact test; $p = 0.28$ by trend analysis.
Fever: CR + PR vs. minor + none $p = >0.20$, by Fisher's exact test

advanced melanoma, including not only subcutaneous and lymph node metastases but such more ominous sites of involvement as the liver and the lung. Treatment was given in an outpatient setting and was generally tolerable even by elderly patients, although side effects and toxicity were undeniably significant, particularly cumulative fatigue and arthralgias. However, most patients were able to go home within an hour after the interleukin-2 infusion was given. Lymphokine activated killer cells were elicited in the circulation of these patients by the in vivo therapy.

The response of melanoma to chemotherapy is 20% at most in large series, and there is no discernible increase in the patient's survival. The regimen we report of low-dose cyclophosphamide and low-dose interleukin-2 appears to have a response rate of approximately 25%, at least equivalent to that found in the expanded trials of interleukin-2 and lymphokine activated killer cells at the N.C.I.[5] and at university centers (Parkinson D., personal communication) (19%). Our rate of remission in melanoma exceeds published rates with high-dose interleukin-2 given without lymphokine activated killer cells (13%).[4] The duration of response to cyclophosphamide plus interleukin-2 was also encouraging, with a median of nine months. In particular, long-term responses of five patients, four of them treated for at least a year, are particularly gratifying. It is worth emphasizing that patients could be treated repeatedly with cyclophosphamide plus interleukin-2, to attempt to improve the degree of response or to maintain stability of the disease at less than 50% of its original volume.

Various sites of disease appear to be differentially sensitive to the effects of interleukin-2, with subcutaneous, lymph node, and pulmonary metastases most responsive in several series.[2,6] The striking response of large liver lesions in four patients was unique in our experience with systemically administered therapy. Two patients with liver involvement responded with complete resolution of substantial lesions, and durable partial remissions were achieved in two others. In contrast, bone and adrenal lesions may prove

most resistant to this form of treatment, judging not only from the lack of responses in these sites, but also from three patients in whom persistent bone pain caused us to stop treatment that had caused regression of disease at other sites, to administer pain-reducing radiation therapy. Even within the same patient we have found one site resistant to treatment while another was responding. In fact, our first patient on study had one lung nodule persist relatively unchanged months after two other lung nodules, skin and liver lesions had all disappeared. It was also not uncommon to find several subcutaneous lesions regressing while others were unchanged. Reasons for this sort of divergent response are not at all obvious, but the phenomenon has also been observed by others with interleukin-2-containing regimens.[6]

The major cause of death in our most successfully treated patients—those who had a complete or partial remission for more than six months—was progression in the brain or spinal cord, a large single metastatic lesion in the brain being the most common presentation. Regardless of its systemic effectiveness, cyclophosphamide plus interleukin-2 cannot prevent or effectively treat lesions of the central nervous system. We are now attempting to devise a means of prophylaxis for the central nervous system in patients with a complete or stable partial remission, but the problem is not easily soluble. High-dose chemotherapy, with autologous bone marrow replacement, is a measure with potential efficacy, but has not yet been tested prophylactically in melanoma.

An improvement in survival cannot be claimed for the entire group, but it is clear that the survival of all but two of the patients with a complete or partial remission was prolonged. In addition, the patient with a long-term, maintained minor response has certainly exceeded expectations for his survival. It was possible to treat one patient intensively for a year and to maintain responses with one two-week cycle every six weeks for 10-18+ months in four other patients.

Among our 10 significant responders were four who had received our regimen of active specific immunotherapy previously,[14] but the other six had never received that therapy. Nevertheless, it will be interesting to combine these two potentially complementary types of treatment, particularly since cytolytic T cells are generated by active immunotherapy and are amplified by interleukin-2, as we demonstrated in two of our patients.

Since lymphokine activated killer cells were elicited solely by in vivo administration of cyclophosphamide and interleukin-2, there seems little

need, at least in melanoma, for ex vivo incubation of peripheral blood leukocytes with interleukin-2. Whether this will be true for such other diseases as renal cell carcinoma and breast cancer, which appear to be sensitive to interleukin-2 and lymphokine activated killer cells, remains to be determined. In any event, a relatively small dose of interleukin-2 has allowed us to treat even elderly patients without medical difficulties of significant proportions, although often at reduced dose levels. However, we should emphasize the necessity for strong psychological supportive care from nurses, physicians, and relatives to encourage all patients to complete the prescribed courses of treatment.

It remains to be proved that cyclophosphamide as we have administered it, in single low doses 3 days before each 2 week cycle, accounted for our therapeutic success with intravenously administered interleukin-2 in the absence of adoptive immunotherapy. Whether our hypothesis for the mechanism by which cyclophosphamide exerted its influence, via the inhibition of suppressor T cells, is correct also requires further study. Our assay for (concanavalin A-induced) suppressor T cells showed no significant change in their activity at 3 and 6 days after cyclophosphamide. Whether interleukin-2 alone would have stimulated higher levels of suppressor cell activity in the absence of cyclophosphamide is uncertain.

Lymphokine activated killer cells are easily measured, and may serve as a convenient criterion for an immunological effect of interleukin-2, but one should not ascribe the in vivo rejection of human tumors uniquely to their activity. Cytolytic and helper T cells, macrophages, and perhaps B lymphocytes may all play important roles. Interleukin-2, which can directly or indirectly influence all of those cells, may cause beneficial responses through effects on several types of cell at once. Nevertheless, lymphokine activated killer cell activation correlated well with a clinical response to therapy, and appeared to be the most important parameter to monitor as a prognostic indicator. Nearly 38% (9/24) of the patients who had an increase in lymphokine activated killer cell activity of at least 10 lytic units per 10^7 mononuclear cells had a remission. More important for prognosis, none of the 14 patients who failed to have an increase in lymphokine activated killer cells in response to interleukin-2 achieved a useful clinical remission.

Ultimately, interleukin-2 will undoubtedly be used as an important part of a combination regimen involving not only other cytokines, but other types of biomodulators and probably chemotherapy as well. There is little doubt that

several regimens involving interleukin-2, including the one we have described, are very useful now in the treatment of melanoma, heretofore a highly resistant form of cancer.

SUMMARY

We have studied the effects of low-dose recombinant interleukin-2 preceded by low-dose cyclophosphamide on malignant melanoma. Thirty eight outpatients aged from 25 to 75 years were treated with interleukin-2, 3.6 million Cetus units/m^2 i.v. daily for five days on two successive weeks beginning three days after 350 mg/m^2 of intravenous cyclophosphamide. This schedule was repeated at least twice more with a one-week interval between cycles, usually at the same dosage level. Ten of the 38 patients (26.3%) had clinically significant remissions: two complete (5.3%), seven partial (18.4%), and one ongoing, long-term (>18 mo) "minor" response (2.6%). Four others (10.5%) had shorter minor responses and four (10.5%) a mixed response. One patient with disease restricted to the skin had a complete remission, while the other patient with a complete remission had had three lung nodules and an enlarged hilar lymph node. It was gratifying that one of the major sites of disease responding to treatment was the liver. Two complete and two partial remissions (i.e., > 50% regressions for > four weeks at this site) were obtained in 10 patients with liver involvement. Lung metastases also responded in four of 16 patients (one complete and three partial remissions). Subcutaneous nodules responded in seven of 21 patients (two complete, five partial remissions), while lymph node metastases diminished significantly in four of 14 patients (one complete, three partial remissions). The median duration of response was nine months (range, 1.5-20 months), with four patients treated for more than one year. Toxicity was moderate and controllable, and only two patients required hospitalization, both overnight. Lymphokine activated killer cell activation was induced in 24 of 38 patients, including all nine of the major responders. Conversely, none of 14 patients without lymphokine activated killer cell activation had a significant clinical remission. This regimen appeared to be as effective in melanoma as those involving ex vivo activation of lymphokine activated killer cells, and was more tolerable than therapy with high doses of interleukin-2.

ADDENDUM

Just after this manuscript was completed, final data became available on the last patient on study. That patient, I.H., a 57-year-old woman who had received no systemic therapy previously, had a 60% regression of disease in

her right adrenal gland, the first patient to respond at that site. In addition, there was a 70% shrinkage of a pulmonary nodule and complete disappearance of breast and ear lobe masses, only a presternal nodule remaining at 24% of its previous size, all as measured by the products of perpendicular diameters. Unfortunately, she had transient amaurosis nearly from the outset, and despite receiving 1.8 million units/m^2 during cycles 2 and 3, the attacks worsened. Toxicity forced us to abandon therapy after only two months. Repeat measurements at all site four weeks after completion of treatment confirmed that partial remission had in fact occurred.

Thus, the final figures in our study are as follows:

Complete remissions	2	(5.1%)
Partial remission	8	(20.5%)
Long-term "minor" response	1	(2.6%)
Minor responses	4	(10.3%)
Mixed responses	4	(10.3%)
No response	20	(51.2%)
Total	39	(100%)

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