# *Clinical and Flow Cytometry Characteristics of Malignant Pleural Effusions in Patients After Intracavitary Administration of Methylprednisolone Acetate*

Arie H. Bartal, MD, DSc,* Yair Gazitt, PhD,† Gamal Zidan, MD,* Bina Vermeulen,* and Eliezer Robinson, MD*

**Ten patients with recurrent pleural effusions due to advanced cancer were treated by intracavitary methylprednisolone acetate (Depo-Medrol [DM], Upjohn, Kalamazoo, MI). They received one to six courses of DM (median, three courses per patient) with doses ranging from 80 to 160 mg per course. Effusion cells were cryopreserved before and during DM installation for subsequent determination of ploidy by flow cytometry. Pleural effusion in all three patients with advanced breast cancer resolved and did not reaccumulate throughout follow-up for 11+, 10+, and 8+ months. Pleural effusion in a patient with metastatic gastric cancer and in two of four patients with adenocarcinoma of unknown origin partially resolved. Altogether six of ten patients (60%) subjectively and objectively benefited from this therapy. All patients tolerated the treatment well with no local or systemic side effects. Flow cytometry showed a reduction in ploidy of effusion cells in all three patients with breast cancer, from a peak mean channel of 6C to nearly 2C after therapy. Transient reduction of ploidy was seen also in the effusion of a patient with unknown primary tumor associated with clinical improvement. The clinical and laboratory data reported offers initial evidence that DM when instilled into the pleural cavity after incomplete thoracentesis may act as effective palliative therapy either alone or in combination with other anticancer agents.**
***Cancer* 67:3136–3140, 1991.**

RECURRENT PLEURAL EFFUSION in patients with cancer causes severe respiratory distress and requires repeated thoracentesis.[1] Although pleural effusion may appear in almost any type of metastatic process, it is most frequently associated with breast and lung cancer. Systemic chemotherapy and/or hormone therapy, determined by the specific type of proliferation, are initiated but often these fail and repeated palliative drainage is needed. Various experimental approaches have been attempted.[2–5] Tetracycline has been introduced into the pleural space after prolonged drainage based on the premise that it may cause local sclerosis, but its efficacy has been marginal.[5–7] Intracavitary administration of chemotherapeutic agents has been used with various responses.[8–13] Recently intracavitary interleukin-2 (IL-2)-activated autologous mononuclear cells have been given combined with systemic IL-2 infusion, and partial responses were induced.[14–16]

Long-acting corticosteroids were injected previously into the pericardial effusion of patients with uremia,[17,18] but to the best of our knowledge, they have not been administered into tumor-associated pleural effusions. We report our preliminary experience with intracavitary methylprednisolone acetate (Depo-Medrol [DM], Upjohn, Kalamazoo, MI) given to patients with recurrent cytologically positive pleural effusion.

Presented at the Second International Conference on Intracavitary Chemotherapy, San Diego, California, February 25–27, 1988.

From the *Hybridoma and Cancer Research Laboratory, Northern Israel Cancer Center, Rambam Medical Center, Haifa, and the †Institute of Oncology, Hadassah Medical Center, Jerusalem, Israel.

Supported by the CONCERN Foundation for Cancer Research, Los Angeles, and the Jane and Harold Hirsh, M.D., J.D., Donation for Cancer Research, Washington, DC.

Address for reprints: Arie H. Bartal, MD, DSc, ImmunoSciences Inc., 160 Community Drive, Great Neck, NY 11021.

Accepted for publication November 15, 1990.

## Materials and Methods

### *Patients*

Ten patients with recurrent pleural effusions due to advanced malignant disease were included in this study. The age, sex, diagnosis, and doses given to these patients are shown in Table 1. No chemotherapy or other hormone therapy was given to the patients during or for 30 days before the administration of DM. All patients had a full clinical checkup before therapy, blood counts, chest radiographs, and bone and liver scans. All patients had positive cytology smears with predominance of malignant cells in the effusion fluid. The protocol included thoracentesis with cryopreservation of effusion cells before and during corticosteroid therapy for subsequent laboratory evaluation.

### *Intracavitary DM Administration*

For the purpose of intracavitary DM administration, the patients were admitted to the hospital initially. Subsequently, when no adverse reactions occurred, outpatient services were used. The initial DM dose was 80 mg, subsequently increased to 160 mg. An 18-gauge needle was inserted into the pleural cavity, and fluid was drained. The volume of fluid removed was 200 to 1000 ml with no effort was made to remove the effusion completely. At the end of the procedure, a bolus injection of DM was given, and the needle immediately withdrawn. In no case was an indwelling catheter left for prolonged drainage because the primary premise was to check the effect of the long-acting corticosteroids on preventing reaccumulation of fluid. Chest radiography was done routinely before and after pleural puncture.

### *Cryopreservation of Effusion Cells*

Cryopreservation of effusion cells before and during DM instillation was done in four patients. Heparin was added immediately to the effusions obtained to prevent clotting. The fluid was then spun down, and the pellet was resuspended in RPMI-1640 culture media (Kibbutz, Beth Haemek, Israel) supplemented with 10% fetal calf serum (Biolab, Jerusalem, Israel) and 10% dimethyl sulfoxide, and frozen at −70°C.

### *Flow Cytometry Studies*

Frozen vials were thawed quickly, resuspended, and washed with phosphate-buffered (PBS) saline twice. The final cell pellet was adjusted to $10 \times 10^6$ cells/ml, and a 0.1-ml aliquot was used for analysis of light scattering by flow cytometry (FACS 400, Becton Dickinson, Mountain View, CA). In parallel, aliquots of $1 \times 10^6$ cells in 0.1 ml PBS were incubated at 4°C for 10 minutes with 1 ml of a solution containing 5 mmol/l $MgCl_2$ and 1% NP40 (Sigma Chemical Co., St. Louis, MO) in 20 mmol/l tris HCl. An aqueous solution of propidium iodide and ribonuclease A were then added at a final concentration of 1 μg/ml and 5 μg/ml, respectively. Cell suspensions were incubated for 10 minutes at 4°C in the dark and analyzed for the distribution of DNA content per cell by flow cytometry at 400 volts and ×4 fluorescence gain. Fluorescence intensity was measured on a linear scale. Ten thousand cells were analyzed in each run. The position of 2C and 4C DNA content per cell was precalibrated by fluorescence beads (Becton Dickinson), and the peak mean channel (PMC) was determined by a built-in computer program.

## Results

The clinical features of the patients and doses given are shown in Table 1. The initial DM dose was 80 mg and was increased subsequently to 160 mg. The patients received two to six courses (median, three courses per patient) given usually on a biweekly basis. The total injected dose per patient ranged from 160 to 820 mg (mean, 420 mg). The therapy was well tolerated with no local or systemic side effects. Pleural effusion in all three patients with advanced breast cancer completely resolved (Figs. 1 to 3). These patients remained free of recurrent fluid throughout subsequent follow-up for 11+, 10+, and 8+ months. The pleural effusions in a patient with metastatic gastric cancer and in two of four patients with adenocarcinoma of unknown origin also responded to drainage and local corticosteroid therapy. Altogether six of ten patients (60%) significantly benefited from this therapy, and their symptoms were relieved.

Samples from three patients with breast cancer and one patient with carcinoma of unknown origin were chosen for careful study of ploidy of the tumor cells in these effusion fluids (Figs. 4 to 6). All three breast cancer patients studied were sampled before and after intracavitary administration of DM. As can be seen in Figure 4 (Patient 2 with breast cancer and a history of other primary tumors, Table 1), the ploidy of the cells decreased progressively from 6C to the normal range of 2C after two courses of treatment. The PMC was reduced from 184 to 59 (Fig. 4, curves 1 to 4). At the same time, light scattering did not change, indicating that we were dealing with a cell population of approximately the same size. Similar results were obtained in the other two breast cancer patients. After the first treatment, Patient 1 showed a shift in PMC from 104 to 47 (4C to 2C), with very little change in light scattering of the cells (Fig. 5). Patient 3 (also ill with breast cancer) had a DNA peak mean channel of 119 before treatment; 2 months after treatment, there was a shift in peak mean channel to 72 (results not shown). However, Patient 4 (with carcinoma of unknown origin) showed little change in DNA PMC after the first course of treatment (Fig. 6A, curves 1 and 2). Curve 3 represents the

TABLE 1. Clinical Features of Patients Treated With Intracavitary Depo-Medrol

| Patient no. | Sex | Age (yr) | Tumor* | Metastasis | Prior treatments | No. of DM treatments |
|---|---|---|---|---|---|---|
| 1 | F | 40 | Breast | PE, LN | S, C, R, H | 2 |
| 2 | F | 66 | Breast, colon, ovary | PE, bone | S, C, R, H | 3 |
| 3 | F | 42 | Breast | PE, bone | S, C, R, H | 1 |
| 4 | M | 72 | CUO | PE, bone | S, C | 5 |
| 5 | M | 70 | CUO, CLL | PE | S, C | 3 |
| 6 | F | 56 | CUO | PE, liver | S, C | 2 |
| 7 | F | 39 | Ovary | PE, LN | S, C | 3 |
| 8 | F | 79 | CUO | BFE, skin | S, C, R | 2 |
| 9 | M | 60 | CUO | PE | S, C | 1 |
| 10 | M | 65 | Lung | PE | S, C, R | 3 |

DM: Depo-Medrol; PE: pleural effusion; BPE: bilateral pleural effusion; LN: lymph nodes; H: hormonal therapy; S: surgery; C: chemotherapy; R: radiation therapy; CLL: chronic lymphocytic leukemia; CUO: cancer of unknown origin.

* Histologic condition in all patients was adenocarcinoma.

second course of treatment where a shift to the left was observed (compare curve 3 with curves 1 and 2). A change in ploidy was also seen after the fifth course of treatment with a shift from PMC of 154 to 88 (Fig. 6B). Despite this temporary improvement based on the ploidy data, this patient was refractory to intracavitary administration of DM.

## Discussion

Although usually considered a separate technique, hormone therapy of cancer can be regarded as a subcategory of biologic-response modifiers. The latter are defined as agents capable of significantly affecting the outcome of a biologic process whether ongoing *in vivo* or *in vitro*. These agents can be induced by exogenous agents (such as bacteria) or endogenously by factors produced physiologically (such as lymphokines).[19] Hormones affect many biologic processes such as growth, maturation, and differentiation of tissues or act on a specific cell population by a unique cellular receptor such as estrogen, progesterone, cortisone, or IL-2 receptors. Corticosteroids are very potent agents, and they alter many biologic responses. Dexamethasone can change organ and cellular growth *in vivo* and *in vitro*.[20] Its effect on lymphocytes is well documented. Stabilization of cellular membranes was reported previously.[21] Due to an antitumoral effect, these agents are being incorporated in combination chemotherapy protocols. Corticosteroids are given orally, intravenously, intralesionally, intraarticularly, intrathecally, and by other routes of administration.[22,23]

Long-acting corticosteroid were previously injected into the pericardial cavity in patients with uremia,[17,18] but to the best of our knowledge, they have not been administered into pleural effusions due to malignant processes.

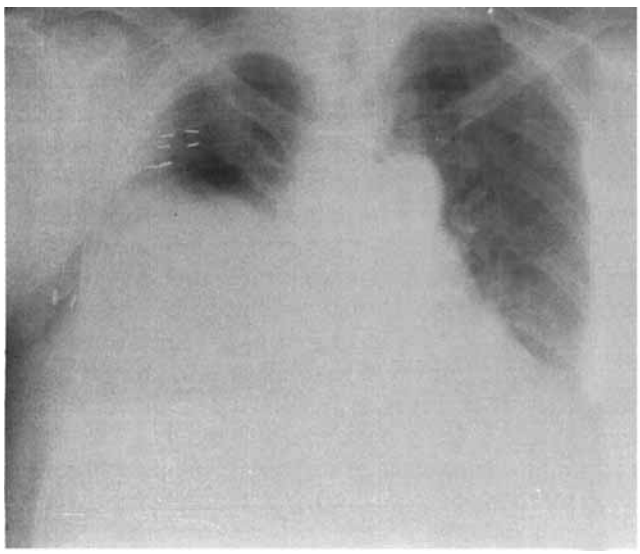


FIG. 1. Chest radiograph showing advanced right pleural effusion in Patient 2 before Depo-Medrol therapy.

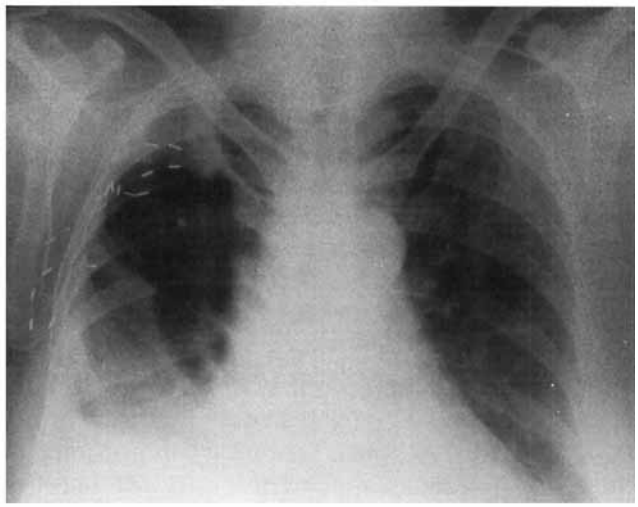


FIG. 2. Chest radiograph after 2 weeks of intracavitary Depo-Medrol therapy, showing significant reduction in fluid. Film was taken before drainage and additional Depo-Medrol injection.

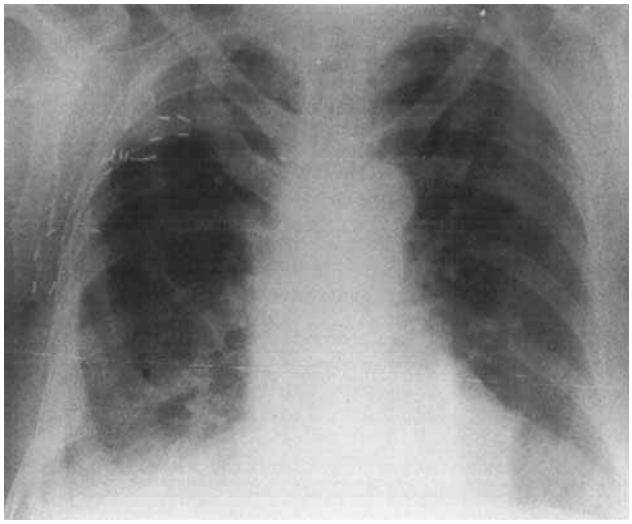


FIG. 3. Chest radiograph 3 months after completion of therapy. This remained practically unchanged during subsequent months of follow-up.

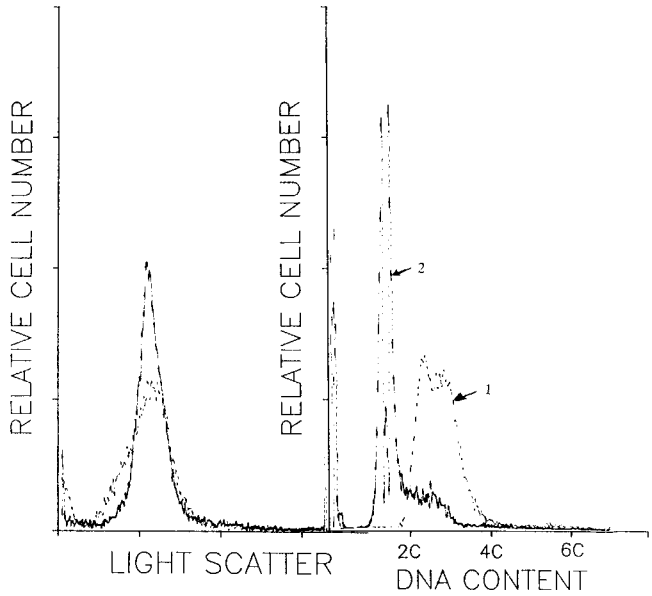



FIG. 5. Analysis of cell size and DNA ploidy of tumor cells from pleural effusions of a breast cancer patient (Patient 1). Curve 1: before treatment, PMC = 89; curve 2: 3 months later, PMC = 47.

We previously observed the potency of corticosteroids when added to hybridoma cells *in vitro*[24,25] and therefore postulated that these agents could have a beneficial effect when given directly into the pleural cavity. Based on the observations made in this preliminary study, there appears to be a significant local effect when long-acting corticosteroids are given by the intracavitary route, the nature of which is not yet clear.[26] These agents may act directly on the tumor cells or through various subsets of reticuloendothelial cells present. An alternative mechanism may be bioalteration of the visceral and parietal layers of the pleural cavity (thus affecting its permeability), but all possibilities may act synergistically.

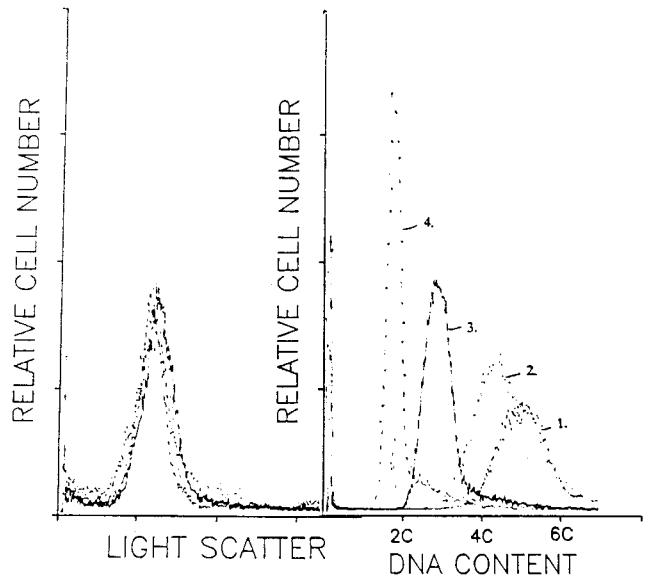
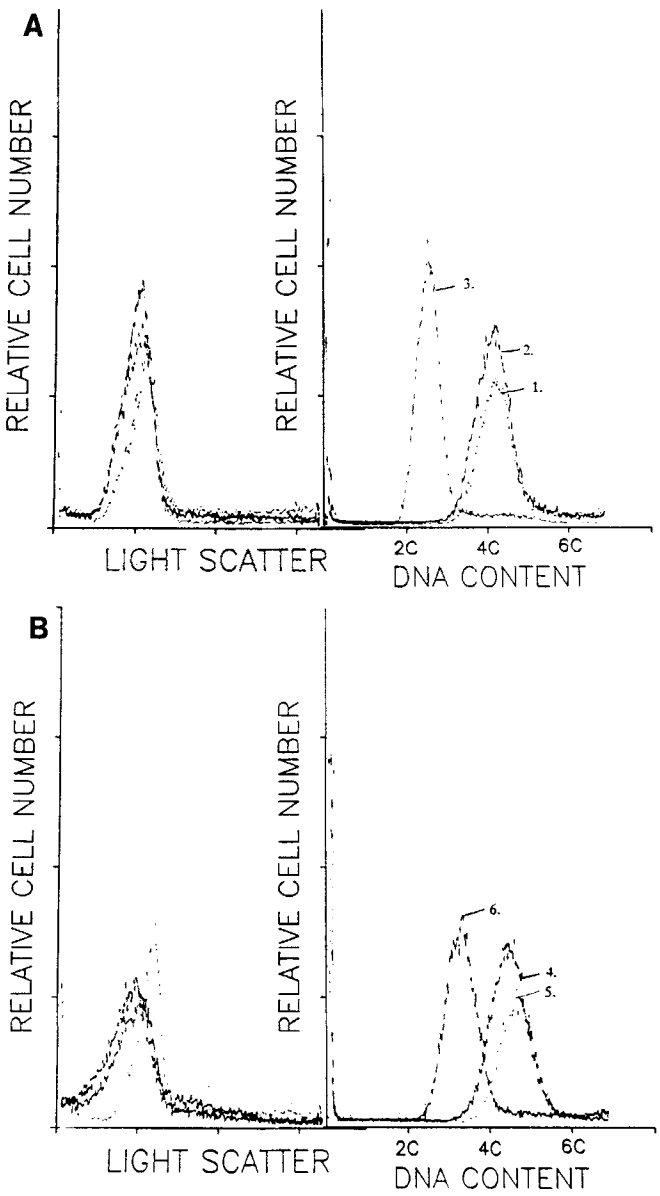



FIG. 4. Analysis of cell size and DNA ploidy of tumor cells from pleural effusion of a patient with breast cancer (Patient 2). Cells were prepared for light scattering measurements and stained for DNA analysis. Experiments were run twice and similar results were observed. Curve 1: before treatment, PMC = 184; curve 2: 2 weeks later, PMC = 160. curve 3: before second treatment, PMC = 89; curve 4: 2 weeks later, PMC = 59. Treatments were given at 4-week intervals.

Light scattering and DNA content distribution analysis in cell populations from the effusion fluids, along with administration of corticosteroids, revealed an interesting close association between clinical improvement and a drastic reduction in the number of tumor cells and cell ploidy. Although the exact count of tumor cells before and after treatment is not available, a rather drastic decrease in cell number and effusion volume was noticed after DM treatment. Ploidy analysis before and during therapy was beneficial, providing a good indication of the efficacy of treatment. Furthermore, it appears that the distribution of the cells with regard to ploidy reflects a strong shift in most cells toward lower ploidy (Figs. 4 to 6). Thus, with regard to the proportion of cells in various ploidy categories, most cells had the same ploidy and type of ploidy. That in all instances there was only one predominant population with one size distribution suggests that the same cells were affected, rather than a change from one population to another. This is supported by the light scattering pattern, both 90° and forward scattering, and by cell morphology.

The parameters we described, together with established clinical workup procedures, may be useful in the rapid evaluation of intracavitary DM treatment and in other experimental approaches. That refractory pleural effusions




FIGS. 6A AND 6B. Analysis of cell size and DNA ploidy of tumor cells from pleural effusions in a patient with carcinoma of unknown origin (Patient 4). (A) Curve 1: before treatment, PMC = 171; curve 2: 1 week after treatment, PMC = 163; curve 3: before second treatment 3 weeks later, PMC = 112. (B) Curve 4: after second treatment, PMC = 154; curve 5: before third treatment, PMC = 153; curve 6: after third treatment, PMC = 88. Treatments were given at 2 to 3-week intervals.

can be partially or completely cleared after administration of these potent hormones is encouraging and should stimulate investigators to look into the factors associated with these phenomena. The clinical and laboratory data reported offer initial evidence that DM, when instilled into the pleural cavity after incomplete thoracentesis, may act as an effective palliative therapy either alone or in combination with other anticancer agents in patients with refractory pleural effusion.

## REFERENCES

1. Anderson CB, Philipott GW, Ferguson TB. The treatment of malignant pleural effusions. *Cancer* 1974; 33:916.
2. Rochlin DB, Smart CR, Wagner DE, Silva RM. The control of recurrent malignant effusions using quinacrine hydrochloride. *Surg Gynecol Obstet* 1964; 118:991.
3. Hancock PM, Hill MW, Johnson NW. The inflammatory response to paraffin in the peritoneal cavity of the rat. *Br J Exp Pathol* 1978; 59: 128–136.
4. Raz A, Shahar A, Goldman R. Characterization of an *in vivo* induced peritoneal macrophage population following intraperitoneal injection of concanavalin A. *J Reticuloendothelial Soc* 1977; 22:445–460.
5. Sahn SA, Potts DE. The effect of tetracycline on rabbit pleura. *Am Rev Respir Dis* 1978; 117:493–499.
6. Robinson R, Bolooki H. Intrapleural tetracycline for control of malignant pleural effusions. *South Med J* 1972; 65:847.
7. Wallach HW. Intrapleural tetracycline for malignant pleural effusions. *Chest* 1975; 68:510.
8. Fracchia AA, Knapper WH, Carey JT, Farrow JH. Intrapleural chemotherapy for effusions from metastatic breast carcinoma. *Cancer* 1973; 31:899.
9. Dedrick RL. Theoretical and experimental bases of intraperitoneal chemotherapy. *Semin Oncol* 1985; (Suppl 4) 12:1–6.
10. Howell SB. Intraperitoneal chemotherapy: The use of concurrent systemic neutralizing agents. *Semin Oncol* 1985; (Suppl 4) 12:17–22.
11. Alberts DS, Young L, Mason N, Salomon SE. *In vitro* evaluation of anticancer drugs against ovarian cancer at concentrations achievable by intraperitoneal administration. *Semin Oncol* 1985; (Suppl 4) 12:38–42.
12. Piccart MJ, Speyer JL, Markman M *et al.* Intraperitoneal chemotherapy: Technical experience at five institutions. *Semin Oncol* 1985; 12:90–96.
13. Markman M, Cleary S, Pfeifle C, Howell SB. Cisplatin administered by the intracavitary route as treatment for malignant mesothelioma. *Cancer* 1986; 58:18–21.
14. West WH, Tauer KW, Yannelli JR *et al.* Constant infusion recombinant interleukin-2 in adoptive immunotherapy of advanced cancer. *N Engl J Med* 1987; 316:898–905.
15. Bartal AH, Birch R, Yannelli JR *et al.* Tumor marker reduction following regional adoptive immunotherapy administration. *Clin Res* 1988; 36:256.
16. Steis R, Bookman M, Clark J *et al.* Intraperitoneal lymphokine activated killer (LAK) cell and interleukin-2 (IL-2) therapy for peritoneal carcinomatosis: Toxicity, efficacy and laboratory results. *Proc Am Soc Clin Oncol* 1987; 6:250.
17. Buselmeier TJ, Simmons RL, Najarian JS, Michael Mauer S, Matas AJ, Kjellstrand CM. Uremic pericardial effusion: Treatment by catheter drainage and local nonabsorbable steroid administration. *Nephron* 1976; 16:371–380.
18. Buselmeier TJ, Davin TD, Simmons RL, Najarian JS, Kjellstand CM. Treatment of intractable uremic pericardial effusion: Avoidance of pericardectomy with local steroid installation. *JAMA* 1978; 240:1358–1359.
19. Oldham RK. Biological response modifiers: Design of clinical trials. *J Biol Response Mod* 1985; 4:117–128.
20. Wells BB, Kendall EC. The influence of corticosterone and C17 hydroxy-dehydrocorticosterone (compound E) on somatic growth. *Mayo Clin Proc* 1940; 15:324–328.
21. Frantz AG. Prolactin. *N Engl J Med* 1978; 298:201.
22. Levine RM, Ramussen JE. Intralesional corticosteroids in the treatment of nodulocystic acne. *Arch Dermatol* 1983; 119:480–481.
23. Nelson LB, Melick JE, Harley RD. Intralesional corticosteroid injections for infantile hemangiomas of the eyelid. *Pediatrics* 1984; 74: 241–245.
24. Bartal AH, Feit C, Hirshaut Y. Modulation of hybridoma formation by dexamethasone. *Nat Immun Cell Growth Regul* 1986; 5:107–112.
25. Bartal AH, Hirshaut Y. Methods of Hybridoma Formation. Clifton, NJ: Humana Press, 1987.
26. Bartal AH, Zidan G, Robinson E. Corticosteroid administration into pleural effusions in cancer patients: A novel and effective therapy. *Proceedings of the American Society of Clinical Oncology* 1987; 6:171.