Seminars in Surgical Oncology 7:199–210 (1991)

Active Specific Immunotherapy and Immunochemotherapy in the Treatment of Lung and Colon Cancer

ARIEL HOLLINSHEAD, PhD, DSc
From the Division of Hematology/Oncology, Department of Medicine, George Washington Medical Center, Washington, D.C.

In this seminar, we describe 1) the immunogen TAA used for lung cancer immunotherapy and the immunogen TAA used for colon cancer immunotherapy, 2) the methods used in the administration of these immunogens in clinical trials of specific active TAA immunotherapy, 3) the results of clinical trials of specific active immunotherapy for lung cancer and for colon cancer patients, 4) the results of immune response monitoring evaluations, and what they indicate, and 5) the way in which certain drugs, selected for their action in the immune system, may be synergistic with specific active TAA immunotherapy, in combination therapy, especially for resected patients of later stages.

KEY WORDS: **active specific immunotherapy, monitoring test, epitopes, immune response, combination immunochemotherapy, synergism between biologic and chemical drugs, helper/suppressor cell changes, methods of administration, tumor-associated antigens**

INTRODUCTION

The surgeon has gained a helpful "extra scalpel," an adjunct to good surgery, a tool which is helpful in cleaning up indetectable cancer cells which remain or which escape during surgery. By adding immunotherapy at the proper time after surgery for cure, or by adding immunochemotherapy at the proper time after cytoreductive or debulking surgery in later stage patients, the chances of longer survival are improved.

Active specific immunotherapy is defined as therapy with immunogens designed to specifically attack the tumor, and to create an active, long-lasting cell-mediated immunity to that cancer. We have reported the clinical and laboratory results of patient studies with active specific immunotherapy and immunochemotherapy using well-defined tumor-associated antigens (TAA), which are gene products of the cancer cells, polypeptides derived and purified from cell membranes and carefully characterized for cancer type, including lung cancers [1–3] and colon cancer [4].

Although the studies of the ways in which the immune mechanisms work in the development or arrest of solid tumors are still in their infancy, we have contributed some new insights into the way in which active specific immunotherapy works, and the ways in which selected drugs and immunogens work together in combination immunochemotherapy. Some of these insights are from clinical measurements and others from laboratory testing of patient materials utilizing sensitive tests developed to measure both humoral and cellular immune responses [2,4].

In this seminar, we will describe separately specific active immunotherapy for lung cancer and for colon cancer: 1) the immunogen, 2) method of administration, 3) results of clinical trials using adjuvant immunotherapy in colon and lung cancer patients, 4) the results of studies of immune responses in these patients and what they indicate, and 5) the way in which selected drugs act upon the immune system when used in combination with immunotherapy.

RESULTS

Lung Cancer

TAA descriptions. Five major types of lung cancer TAA biologic drugs/immunogens were prepared. Tests

Address reprint requests to Ariel Hollinshead, Ph.D., D.Sc., Division of Hematology/Oncology, Department of Medicine, George Washington Medical Center, 2300 I Street NW, Washington, DC 20037.

in patients to compare single and combination TAAs permitted the selection of the strongest synergistic effect per given protein concentration. The TAA immunogens were prepared in batches with good reproducibility between batches, and with careful quality control. The contents for each lung cancer type are described in Table I.

Note that some antigens are shared between these strictly evaluated histologic types. Yesner and Hollinshead [4] have reported the substantiation of the "Y-theory" of the origination of lung cancers, since the patterns of the above TAA biomarkers coincide remarkably with the postulation that all lung cancers have a common origin in the endoderm. As supported by other evidence, the Y concept has small cell carcinoma at the base of the Y, large cell carcinoma at the fork, and at each arm are squamous cell carcinoma and adenocarcinoma. Note the pattern of shared antigens amongst the TAAs as consistent with this theory. Synergistic TAAs of bronchioalveolar carcinomas include 77 kDA marking this subclass of adenocarcinoma, and 100 kDa as shared with squamous cell carcinomas, perhaps related to the strong keratin expression of pneumonocytes.

Administration of immunotherapy. TAA immunogens are well-homogenized with adjuvant and administered intradermally once per month for 3 months. The first immunization is given about 2 weeks after surgical removal of lung tumors. The principle of active specific immunotherapy is based upon the well-described, classic dual effect of appropriate immunization. Proper emulsification of antigen and adjuvant is crucial. We have used 500 μg TAA immunogen in 0.2 ml emulsified with an equal volume of complete Freund's adjuvant, but have learned that a good emulsification can be obtained using 0.1 ml adjuvant, and that 300 μg TAA per 0.2 ml is adequate. We usually administer the emulsified immunogen in three adjacent sites, a triangle formation, on one arm the 1st month, the other arm the 2nd month, and on the thigh the 3rd month. Physicians are warned that any usage of steroids may interfere with the efficacy of this therapy. The first action induced by immunization is an immediate release of the aqueous portion of emulsion, followed by boosting from the emulsified portion. Classic studies show that a well-prepared immunogen resides at the site of injection for several months, is constantly transported to subpleural parts of lungs and is detected not only in regional but also distant lymph nodes. So far, other adjuvants have not been shown to induce long-lasting cell-mediated immunity, the keystone of classic active specific immunotherapy.

Responders to immunotherapy mount specific immune responses which last even as long as 12–14 years. Toxicity includes small skin ulcers at the sites of immunization, similar to that seen for smallpox vaccinations, and these generally heal within 4 to 6 months; a possible overnight fever may occur, although emulsification reduces the tendency toward febrile reactions. There are no long-term toxic effects, specifically no autoimmune reactions, using these highly purified polypeptides, essentially free from nucleic acids, major tissue antigens, pyrogens, bacteria, and viruses.

Clinical studies. After a successful phase II clinical trial in stage 1 lung cancer patients, two phase III clinical trials in stages 1 and 2 disease were conducted. Each of these trials have been reported separately, and are described in detail in well-referenced overall reports [1–3]. Except for the inclusion of stage 2 disease in the phase III trial, the common features of the three clinical trials were the same. The selection criteria included no previous treatment prior to curative resection; no history of other malignancy in the past 5 years; contraindications were pregnancy or stages 3 and 4 disease; stage 1 and 2 disease fully resected with all excised lymph nodes diagrammed and numbered. Staging criteria included surgical and pathology reports, American Joint Committee TNM staging, as well as a final overview of pathology with review of records and slides and final staging determination. The eligible patients were strictly randomized into control and therapy groups, with the follow-up to be after 5 years. The actual 5-year survival is shown in Figure 1. Distribution according to stage, including two patients with T2N2 initially staged incorrectly, are as shown in Table II. *P* values as assessed by both Wilcoxon and Savage tests were all below .00. Kaplan-Meier density analyses were highly significant. Optional studies in individual trials included an analysis of a group using adjuvant alone, and no statistical difference from the control group was seen at 5 years.

Immune changes. We have reported in detail [2] measurements of *cell-mediated immunity* and measurements of *humoral immunity* by controlled, standardized tests. There was a striking correlation between patients with long-lasting CMI and strong early humoral responses to immunotherapy. The latter test of HI em-

TABLE I. Lung TAA Immunogens Used for Treatment in Each Type of Lung Cancer

TAA immunogens	Molec. weight	Description
Oat cell	51 kDa	Unique protein[a]
	69 kDa	Unique lipoprotein
Large cell	37 kDa	Unique protein
	51 kDa	Unique protein
Squamous cell (epidermoid)	37 kDa	Unique protein
	49 kDa	Fetal protein
	100 kDa	Fetal lipoprotein
Adenocarcinoma	51 kDa	Unique protein
	77 kDa	Unique protein
Bronchioalveolar	77 kDa	Unique protein
	100 kDa	Fetal lipoprotein

[a]Not found in this form in normal lung.

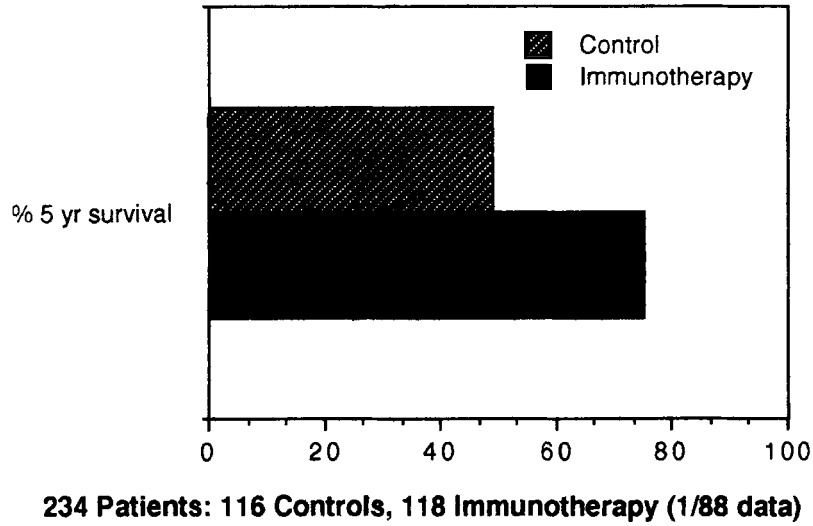


Fig. 1. Lung cancer specific active immunotherapy—stages 1 and 2.

TABLE II. Distribution According to Stage of 234 Lung Cancer Patients in Lung Cancer Immunotherapy Trial

	Controls	Immunotherapy
T2N2	0	2
T2N1	17	22
T1N1	9	13
T2N0	43	44
T1N0	47	37

ployed a reverse enzyme immunoassay utilizing serial sera from patients before, during, and after immunotherapy versus purified, separated, well-characterized TAA epitopes. This assay appears to discriminate between patients who respond to therapy and those who fail therapy 5–6 months after commencement of immunotherapy. This will permit us to try other therapies in such patients, with options such as combination immunochemotherapy, booster TAA shots, additional polyvalent vaccines, and other strategies, since there is still time to try different approaches. The tests of HI also revealed other useful data. In our first clinical trial, we had tested a small group with combination methotrexate plus TAA immunotherapy versus methotrexate alone.

Although the numbers were too small for statistical evaluation, in our studies with the HI assay, we identified a cohort of patients with adenocarcinomas who appeared to respond to combination chemoimmunotherapy with earlier and stronger specific HI. These data indicate the importance of a larger trial in this subgroup to test the possible efficacy of combination therapy.

Combination immunochemotherapy. In addition to the above observations, it may be useful to expand the clinical studies of lung cancer patients to include patients with later stage disease where cytoreductive surgery is feasible, followed by carefully selected immunochemotherapy. Neither chemotherapy or immunotherapy of solid tumors has been shown to have a predictable effect upon B and T cells or subsets in patients with solid tumors, especially when measured within the first half year of therapy. However, we have observed that the combination of both therapies, using drug and dosage carefully chosen for effect upon the immune system, may have a dramatic effect in resected later stage patients. An example is shown in Table III.

Note that the effect of methotrexate, as we have reported previously, is to cause a rebound overshoot, to flush-up the white cells. Of great interest is the fact that in this patient there appeared to be a large component of occult suppressor cells which made up the bulk of the cell overshoot response. The use of TAA specific active immunotherapy, when given at the proper time, at the

TABLE III. Helper/Suppressor Cell Changes During Course of Treatment of a 60 Year Old Female With Adenocarcinoma of the Lung

	Adenocarcinoma of the lung (60F, multiple metastasis: immunochemotherapy)		
Dates of therapy and measurements	CD4/CD8	CD4	CD8
6/13 (pretherapy)	2.71	8.19	3.02
8/18–19 (methotrexate + citrovorum rescue)			
8/22	0.21	7.0	33.0
8/23 (300 μg TAA + FCA = 0.3 ml)			
9/12	3.24	8.47	2.61
9/19 (TAA + FCA)			
10/10 (22 days postmethotrexate)	6.36	9.34	1.46
10/17 (TAA + FCA)			
12/14 multiple metastases disappeared			

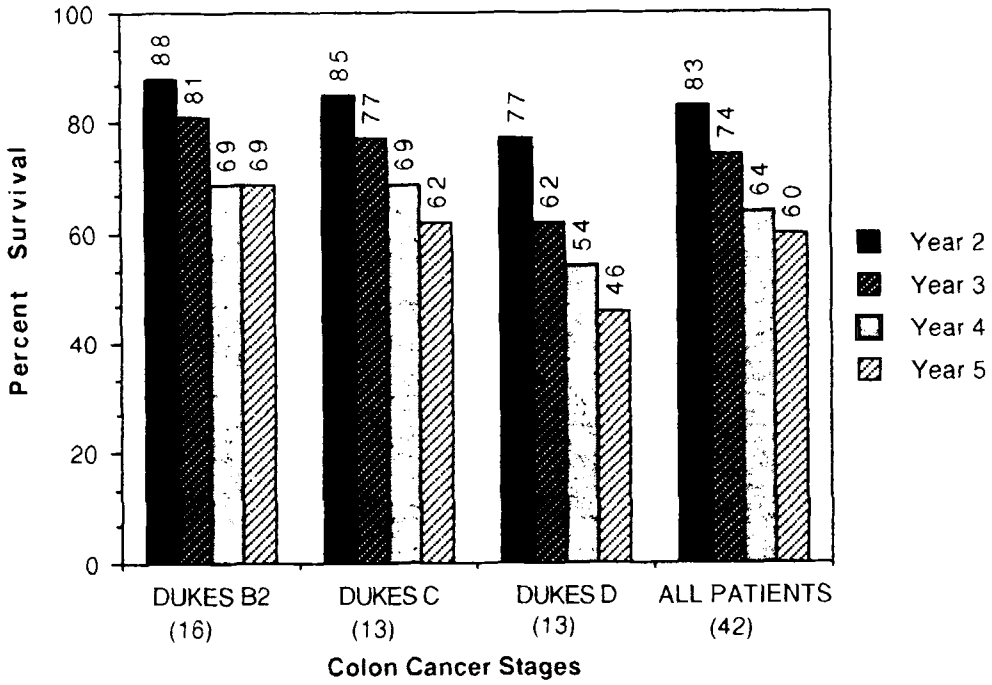


Fig. 2. Specific active colon cancer immunotherapy: 5 year survival.

peak of the overshoot, "educated" the flushed-up cells, and in combination with the drug sustained and restored the helper cells to an improved balance. Clinical effects of the combination therapeutic strategy followed.

Colon Cancer

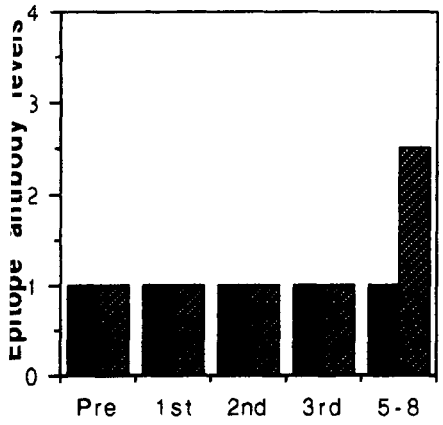
TAA description. As reported elsewhere [5], colon TAA consists of two synergistic polypeptides with molecular weights 72 kDa and 88 kDa. Each polypeptide is biologically active for CMI as measured by delayed hypersensitivity skin tests and specific lymphocyte stimulation. TAA is weakly present in some tumors and strongly present in others. The purification, separation, and preparation of quality controlled batches assures predictable, reliable biologic drugs. Some metastatic tumors have altered or non-existent primary 88 kDa TAA, and an altered or new antigen which induces CMI is identified between 85 and 89 kDa.

If later stage colon cancer patients are to be treated, this variant is included in the immunogen. Colon TAA utilized at low concentrations stimulates a dose-dependent, sensitive, and selective lymphocyte blastogenic response in antigen-primed human splenocytes.

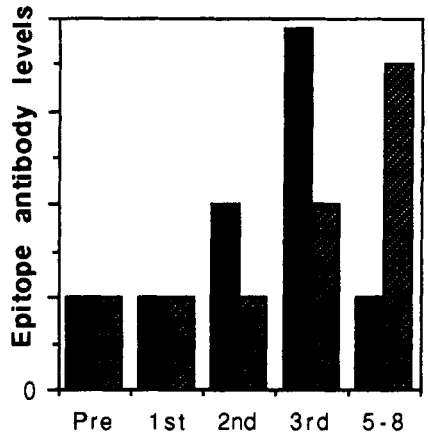
Administration of immunotherapy. The methods of administration of colon TAA specific active immunotherapy are similar to that described for lung cancer patients, except that the administration is given in the thigh the 1st month, in the alternate thigh the 2nd month, and in the arm the 3rd month. Three hundred micrograms colon TAA per 0.2 ml is well emulsified with 0.1 adjuvant and slowly innoculated intradermally in three adjacent sites.

Clinical studies. Phase I/II studies have been completed and reported [5]. A total of 42 patients have received immunotherapy, with 28 of these patients extensively evaluated by laboratory and clinical tests for safety in terms of toxicity, and all 42 evaluated for safety in terms of survival. Dukes stages B2, C1, C2, and D were entered. No autoimmune reactions were seen, specifically no enteritis. There was no clinical or biochemical evidence of toxicity other than local skin ulceration at the ID injection site, and these healed in 4–5 months. Sex distribution was 25 males and 17 females; age range was 25–72 years (median 58.5 yrs). Mean survival of 42 patients was 64.9 months; 25 of 42 patients survived

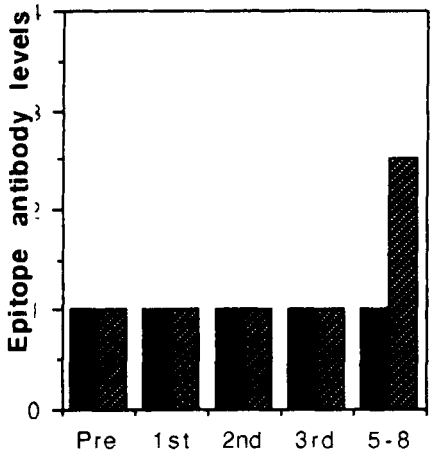
Fig. 3. Charts of colon cancer patient immunoassays. As shown on the **vertical axis** of each chart, epitope protein concentrations reacting with patient serums were measured. In order to depict the data in graphic form, a concentration of 300 pg (negative value) was given a value of 1.0. The 200, 150, 100, 50, and 10 pg concentrations were given the values of 2.0, 2.5, 3.0, 3.5, and 3.9, respectively, the latter value being the lowest epitope protein concentration (10 pg) which reacted with the patient serum. The serial sera from each patient are shown on the **horizontal axis:** Pre = Pre-immunization; 1st, 2nd, 3rd = the 1st, 2nd, and 3rd months of TAA immunizations; 5–8 = sera taken 5–8 months after immunotherapy and measured as months since the first immunization. The **dark** columns show the measure of epitope 68d-4 and the **hatched** columns show the measure of epitope 68d-1. Note: Sera were not available for patients 1 and 10 (exp = expired; lfu = lost to follow-up; ned = no evidence of disease).



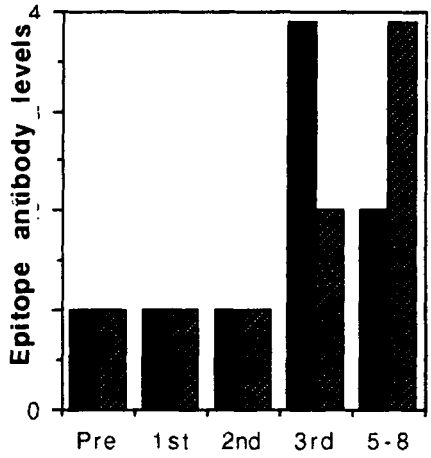
Patient 2
Dukes B2, survival 36 mo, lfu



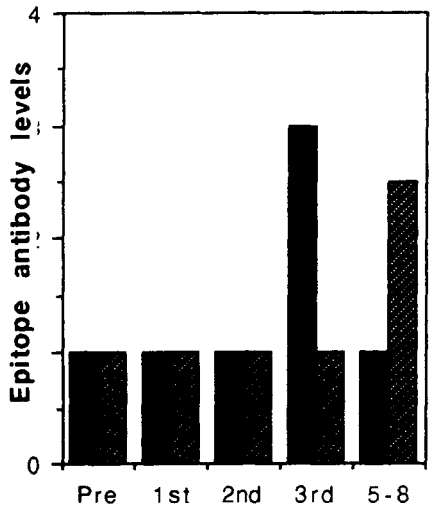
Patient 3
Dukes B2, survival 102 mo, ned



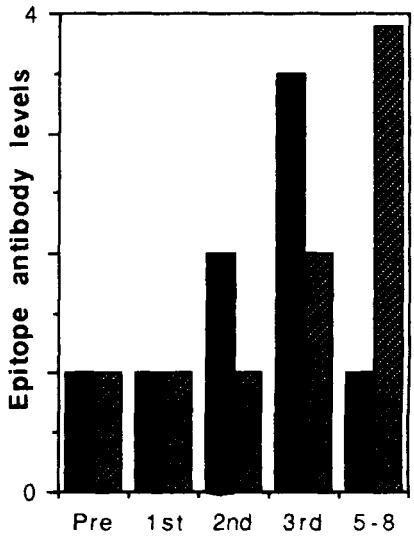
Patient 4
Dukes B2, survival 33 mo, exp



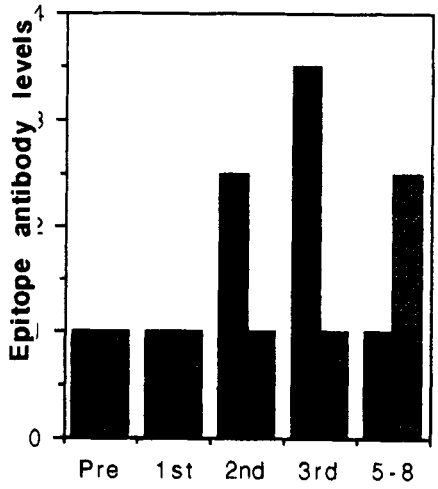
Patient 5
Dukes B2, survival 83 mo, ned



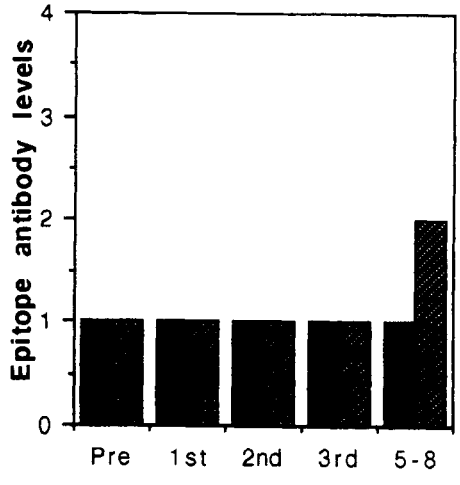
Patient 6
Dukes B2, survival 83 mo, exp



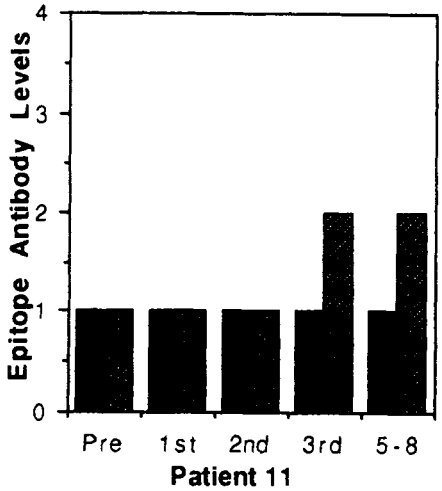
Patient 7
Dukes B2, survival 69 mo, ned



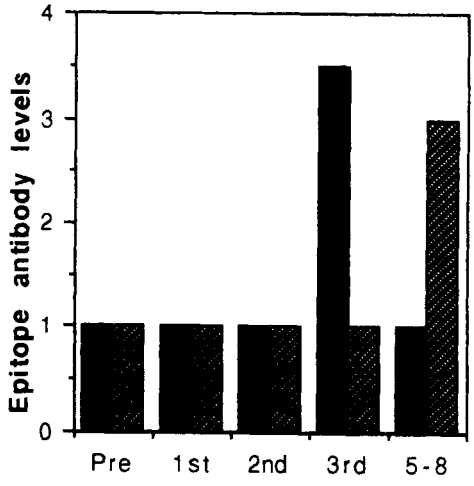
Patient 8
Dukes B2, survival 80 mo, lfu



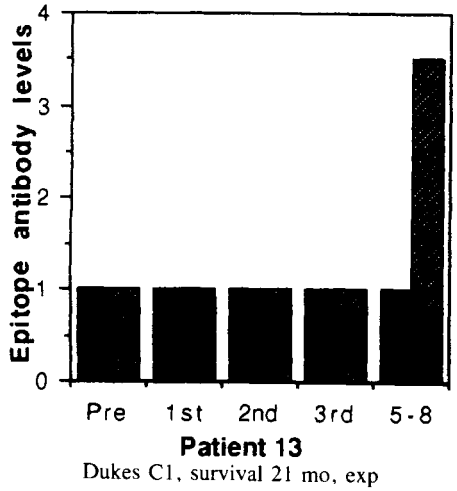
Patient 9
Dukes B2, survival 36 mo, exp



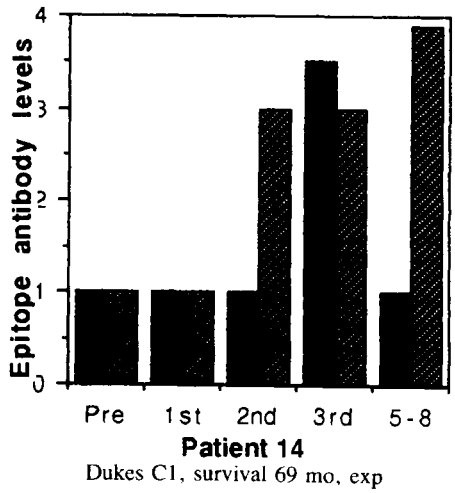
Patient 11
Dukes C1, survival 50 mo, exp



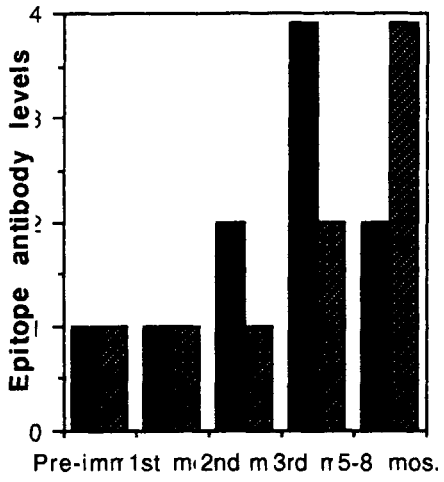
Patient 12
Dukes C1, survival 23 mo, exp



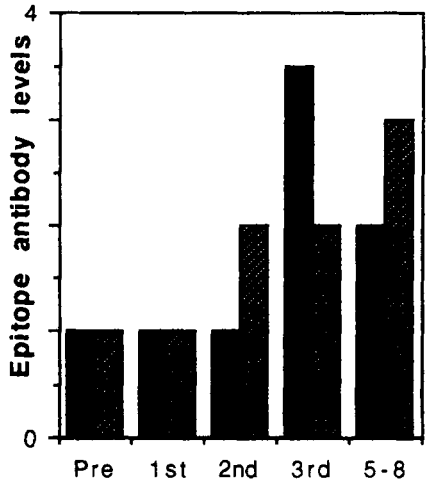
Patient 13
Dukes C1, survival 21 mo, exp



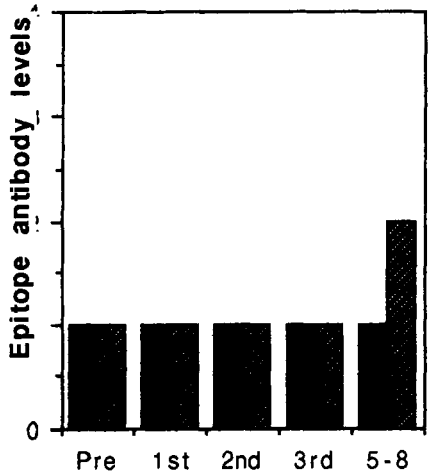
Patient 14
Dukes C1, survival 69 mo, exp



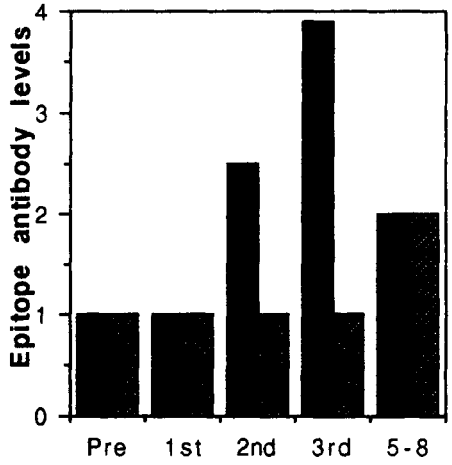
Patient 15
Dukes C1, survival 101 mo, ned



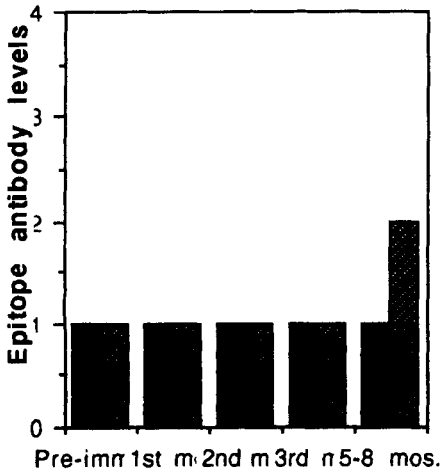
Patient 16
Dukes C2, survival 77 mo, ned



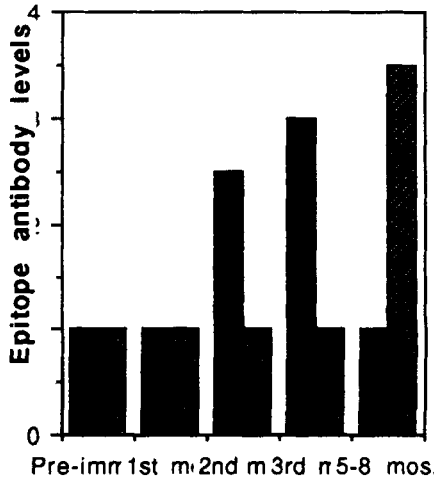
Patient 17
Dukes C2, survival 24 mo, exp



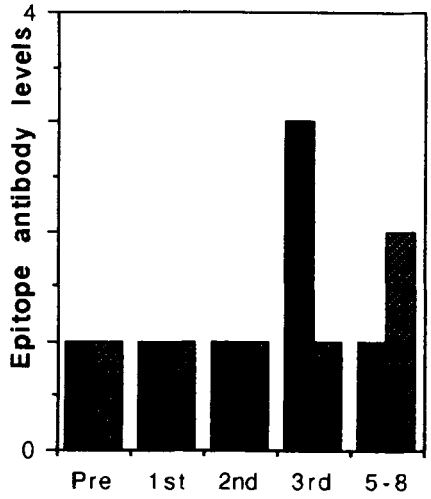
Patient 18
Dukes C2, survival 146 mo, ned



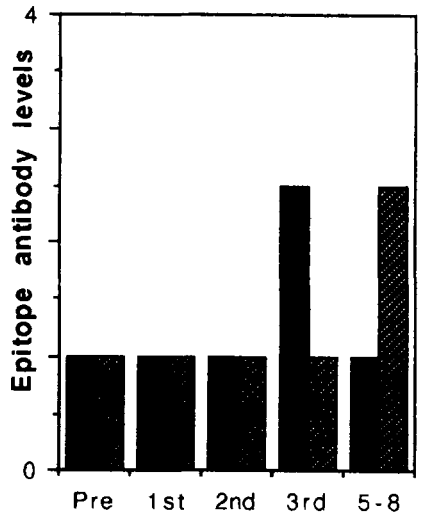
Patient 19
Dukes C2, survival 45 mo, exp



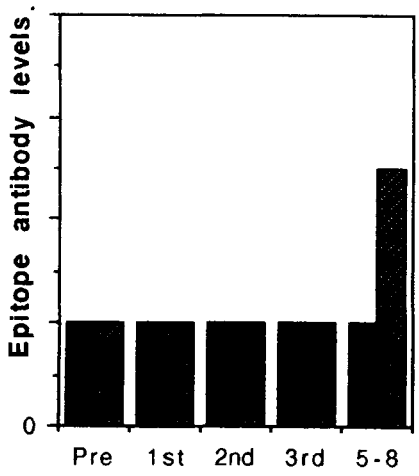
Patient 20
Dukes D (solitary liver metastasis), survival 91 mo, exp



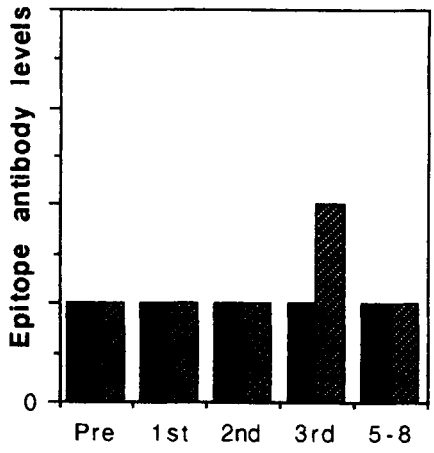
Patient 21
Dukes D (solitary liver metastasis), survival 82 mo, exp



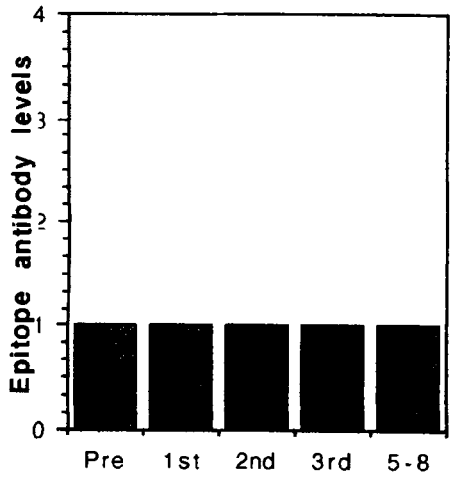
Patient 22
Dukes D, survival 72 mo, exp



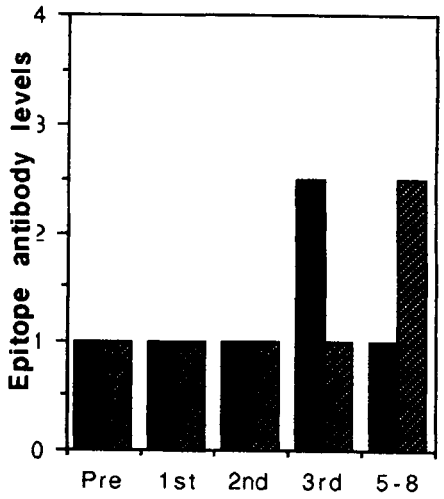
Patient 23
Dukes D (solitary liver metastasis), survival 55 mo, exp



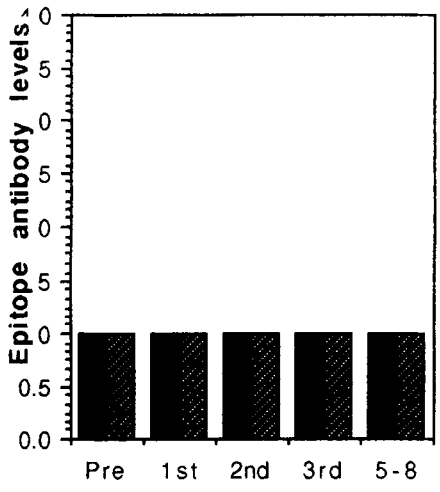
Patient 24
Dukes D (diffuse liver metastases), survival 33 mo, exp



Patient 25
Dukes D (peritoneal studding), survival 18 mo, exp

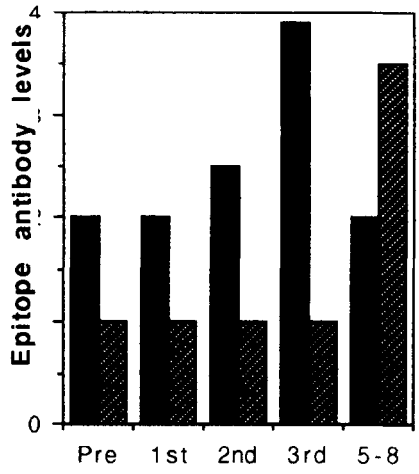


Patient 26
Dukes D (sacrum, small bowel, and Gerota's fascia), survival 61 mo, exp



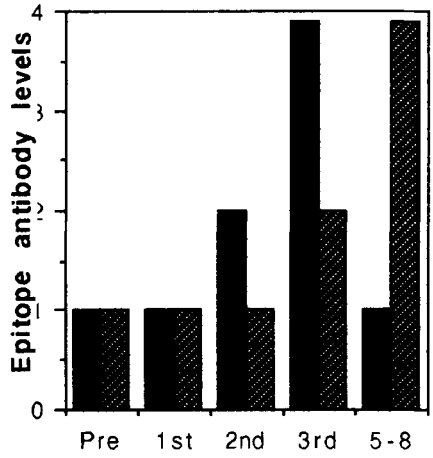
Patient 27

Dukes D (large para-aortic recurrent mass), survival 23 mo, exp



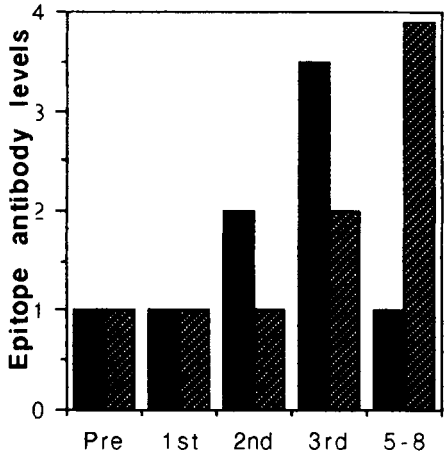
Patient 28

Dukes D (attach. renal capsule), survival 156 mo, ned



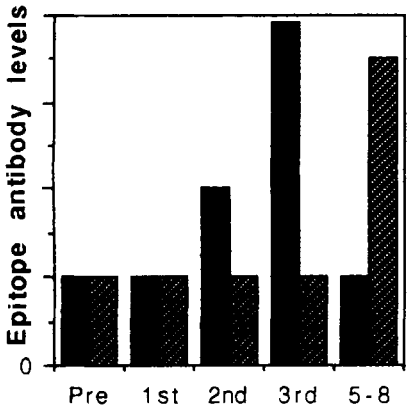
Patient 29

Dukes B2, survival 146 mo, ned



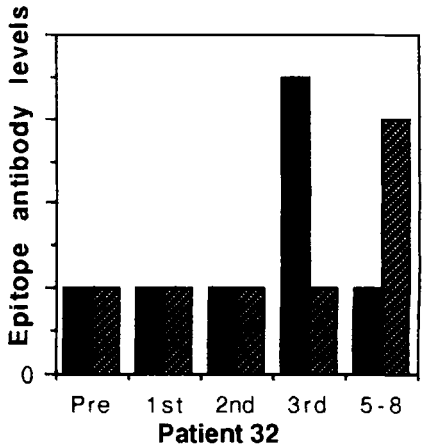
Patient 30

Dukes B2, survival 118 mo, ned



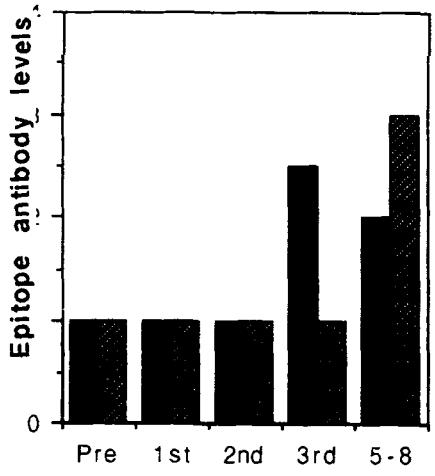
Patient 31

Dukes B2, survival 105 mo, ned

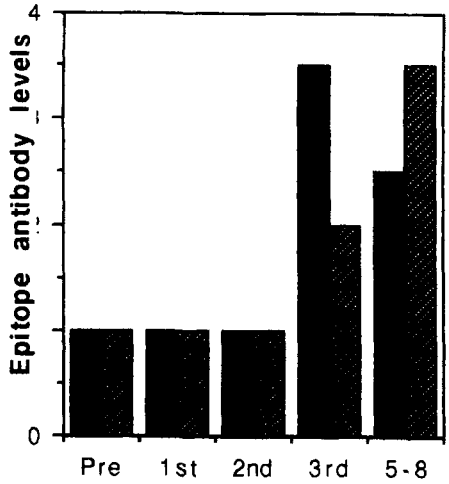


Patient 32

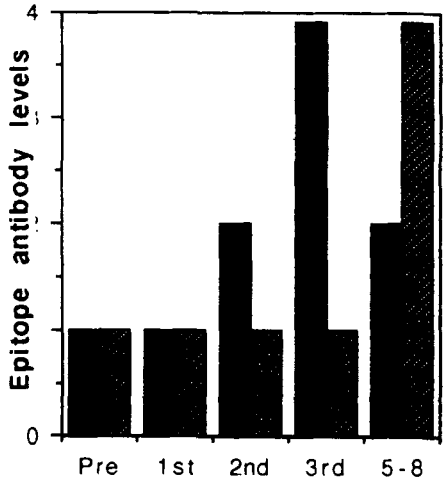
Dukes B2, survival 69 mo, exp



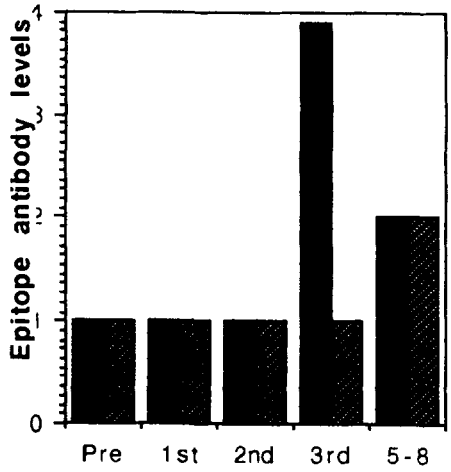
Patient 33
Dukes B2, survival 63 mo, lfu



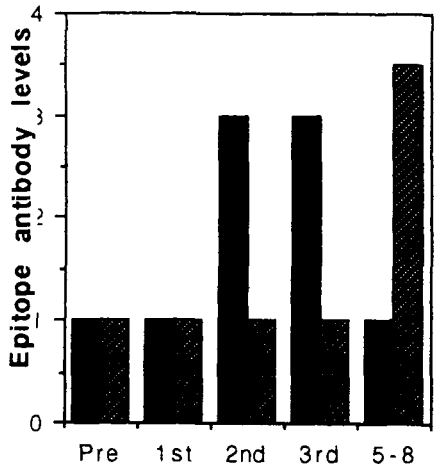
Patient 34
Dukes B2, survival 61 mo, ned



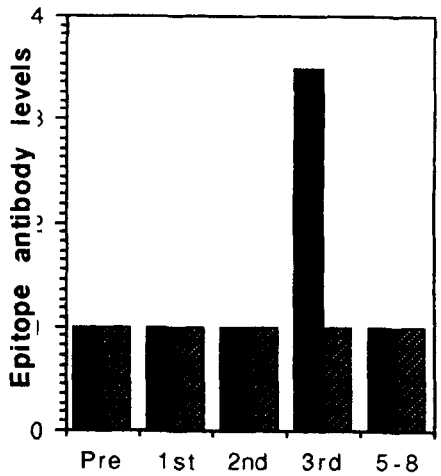
Patient 35
Dukes C1, survival 96 mo, ned



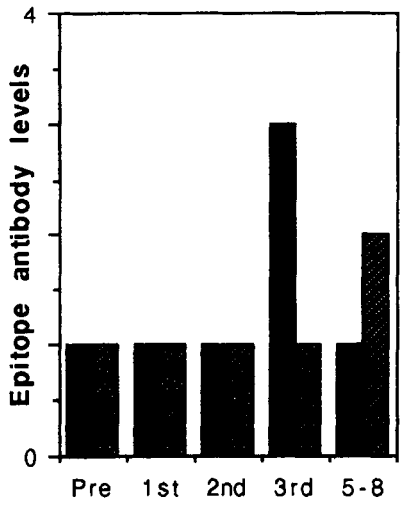
Patient 36
Dukes C2, survival 78 mo, ned



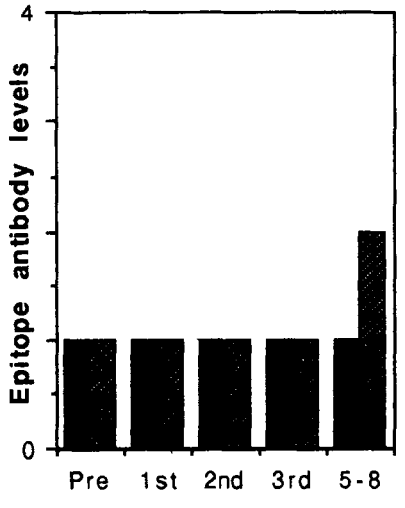
Patient 37
Dukes C2, survival 69 mo, ned



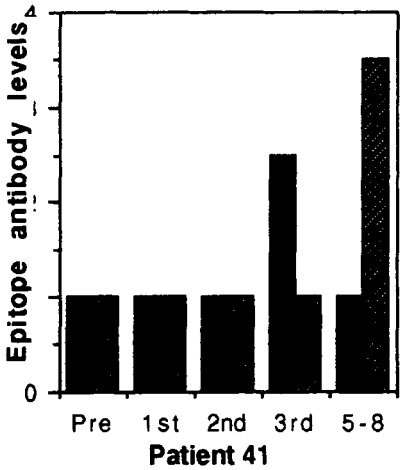
Patient 38
Dukes C2, survival 62 mo, exp



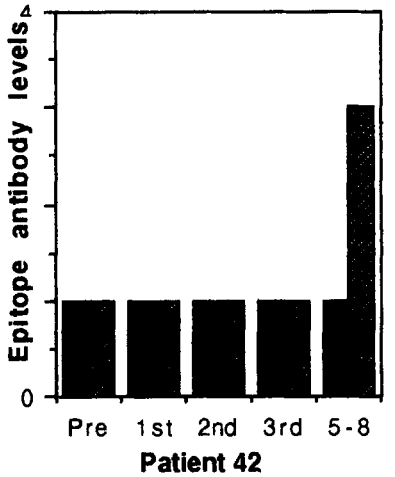
Patient 39
Dukes D (lung lesion disappeared), survival 62 mo, exp



Patient 40
Dukes D (cancer of the transverse colon, invading the mesocolon as a mass), survival 34 mo, exp



Patient 41
Dukes D (recurrent in pelvis), survival 22 mo, exp



Patient 42
Dukes (solitary liver metastasis), survival 47 mo, lfu

longer than 5 years. Results of 12/89 are shown in Figure 2.

Immune changes. Serial sera from 40 of the 42 patients (sera from patients number 1 and 10 were not available) were tested in coded, controlled, and standardized titration reverse enzyme immunoassays in triplicate. These results are reported in detail [5], and are presented here in chart form for easy clinical evaluation. The sera were tested against colon cancer epitopes 68d-4, which is the most important evaluation, since it appears to measure earlier TAA antibody levels and is predictive of long survival, and 68d-1, which measures later antibody levels, although still within the 1st year, and is reflective of an unknown cell response. These data indicate that in colon cancer patients there is at least a postimmunization bi-level antibody response. In the Figure 3 legend, we give the interpretation of the data and patient information. Careful study of the charts and data will provide the physician with a rapid ability to evaluate the usefulness of this monitoring test. Although, in general, patients with colon cancer not on specific active immunotherapy do not appear to mount a correct or strong immune response without appropriate therapy, there is one patient out of the 40 measured here in which a correct immune response is present and in whom TAA immunization augments this response (see patient 28). If one stretches the statistical probability, this means that no more than 2 patients out of 40, or 1 in 20, should receive any form of nonspecific immune stimulation, and it would be hard to predict which patient might be eligible. In clinical trials with substances like levamisole, an unknown immunogen, or interferon, 19 out of 20 patients might have an augmentation of the wrong immune response, whilst 5% of patients might benefit, and the overall survival rates would reflect this effect. We suggest that the data here may be very helpful in alerting the physician to such

TABLE IV. Helper/Suppressor Cell Changes During Course of Treatment of a 64 Year Old Female With Adenocarcinoma of the Colon

Dates of therapy and measurement	Adenocarcinoma of the colon (64F, Dukes Stage D: immunochemotherapy) CD4/CD8	CD4	CD8
3/2 (pretherapy)	2.26	7.12	3.15
3/11 (Continuous intravenous infusion of 5-FU, 30 mg/kg for 5 days)			
4/1	3.11	6.41	2.06
4/1 (300 μg TAA + FCA = 0.3 ml, intradermally)			
5/6 (300 μg TAA + FCA = 0.3 ml, intradermally)			
6/1 (300 μg TAA + FCA = 0.3 ml, intradermally)			
8/6	5.95	9.58	1.61
8/6 Total regression of liver metastases			

possibilities, which require strict verification at the clinical level.

Combination immunochemotherapy. Seven Dukes D patients received combination therapy consisting of 5-fluorouracil and colon TAA immunotherapy. 5-FU has a very different mode of action upon the immune system, and was selected for its effect upon suppressor cells. As shown in the example (Table IV), there is an effect by the drug in cutting the number of suppressor cells by one-third. The follow-up synergistic action of the immunotherapy upon the cell-mediated immune responses permits an effect of the immunochemotherapy upon the cells and a return to a more healthy ratio of helper to suppressor cells. The patient chosen in this example is of a similar age, sex, and stage so that a comparison may be made with the effects of methotrexate combination therapy in the lung cancer patient example (Table III).

REFERENCES

1. Hollinshead A, Stewart THM, Takita H, Dalbow M, Concannon J: Adjuvant TAA specific active immunotherapy trials. Tumor-associated antigens. Cancer 60:1249–1262, 1987.
2. Hollinshead A, Takita H, Stewart T, Raman S: Specific active immunotherapy. Immune correlates of clinical responses and an update of immunotherapy trials evaluations. Cancer 62:1662–1671, 1988.
3. Hollinshead A, Takita H, Stewart T: Review of experience in clinical trials of specific active tumor-associated antigen immunotherapy of lung cancer. In Schirrmacher V, Schwartz-Albiez R (eds): "Cancer Metastasis." Heidelberg: Springer-Verlag Publ., ISBN50471-0 Proj. -Nr. 27173-2, 1989.
4. Yesner R, Hollinshead A: Immunopathology and the natural history of lung cancer. Proc AACR 30:222, Abstr. 884, 1989.
5. Hollinshead A, Stewart THM, Elias H, Arlen M: Co-assessment of serum epitope antibodies, cell-mediated immunity and survival in colon cancer patients on TAA specific active immunotherapy. pp 454–468. In Salmon SE (ed): *"Proceedings of the Sixth International Conference on the Adjuvant Therapy of Cancer,"* Chapter 56. Philadelphia: W.B. Saunders Inc., 1990, 454–468.