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Adjuvant Specific Active Lung Cancer Immunotherapy Trials

Tumor-Associated Antigens

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The 10-year cumulative experiences of five year survivals of patients entered into a successful phase II specific active tumor-associated antigen (TAA) immunotherapy trial, a successful phase III specific active immunotherapy trial A and of patients from centers with acceptable protocol violation levels of an unsuccessful specific active immunotherapy trial B are evaluated. Here the authors report the efficacy of specific active TAA immunotherapy when the protocol is adhered to strictly, where the induction of cell-mediated immunity to TAA indicated a successful adherence to the protocol rather than the strategic result when centers from the third trial with major violations are included. The authors repeat here a summary of each of the three separate trials, each of the three trials having been reported elsewhere in their entirety, so that these total results may be compared to the present analysis. The survival experiences of a total of 234 lung cancer Stage I and Stage II patients, including all violations, from centers in northern New York, northern New Jersey, western Pennsylvania and eastern Canada show a statistically valid ($P = 0.0002$) 5-year survival difference between the control groups (receiving adjuvant alone or no treatment) at 49% survival and the specific active immunotherapy groups at 69% survival. The best promise of specific active immunotherapy alone in an adjunctive treatment setting is with early stage lung cancer. In addition to tests which monitor the effect of TAA immunotherapy induction of long-lasting cell-mediated immunity, tests (monoclonal antibody-derived epitope enzyme immunoassays) were developed to monitor specific, early antibody rises in the bloodstream (circulating humoral immunity).

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In THIS ARTICLE we present the results of multicenter trials using a single approach to test the efficacy of polypeptide tumor-associated antigens (TAA) as adjuvant specific active immunotherapy. In addition, we describe hybridoma-monoclonal antibody-affinity chromatography selection of TAA polypeptide subunits as antigenic determinants (TAA epitopes) for use in monitoring, early on during the course of treatment, the effect of specific active immunotherapy. Now that biologic drug TAAs have been tested as a single entity in well conducted, randomized, controlled clinical trials, this single form of therapy selectively may be combined either with drugs or with biologic drugs in future studies.

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Background

Trial 1: Specific Active Immunotherapy Phase II Trial

We have reported previously a phase II clinical trial[1,2] which was conducted to evaluate therapeutic efficacy of specific active TAA immunotherapy in patients with Stage I lung cancer. As shown in Figure 1, 52 patients were tested and were randomized to 16 controls, eight of whom received methotrexate alone, 15 of whom received immunotherapy alone, and 13 of whom received immunotherapy plus methotrexate. As in the other trials,[3–5] patients were given soluble TAA well homogenized in Freund's complete adjuvant (FCA), once per month for 3 months. The median age of the patients was 56 years; sex distribution was 39 men and 13 women. All were surgically resected by the same surgeons. The major objectives of this trial were to see whether specific immunization would induce a strong delayed hypersensitivity reaction to TAA, and to compare the disease-free

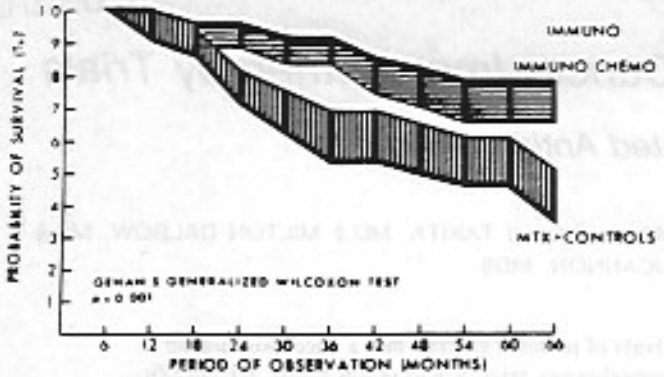


FIG. 1. Phase II specific active immunotherapy trial, September 1, 1980, Stage I lung cancer. This is a graphic representation of the two survival curves. A significant separation of the curves is seen beyond 2 years to 5.5 years. (Figure courtesy of The Yale J. Biol. Med. Inc.)

interval and/or survival of immunized patients with untreated control patients.

In the phase II clinical studies, no autoimmune reactions were observed after TAA immunotherapy. Specifically, the patients had no pneumonitis. As summarized in Figure 1, the 5-year survival of the immunized group was 78% and the 5-year survival of the control group was 46%. No statistical differences in survival were seen in the drug alone group and the control group so these groups could be combined, as reported.[1,2] The difference in survival was examined by two-sided statistical analysis of the 95% confidence limit. Significant differences in survival (upper and lower curves) at the 95% confidence level (shaded areas) were noted at 18 months and beyond (Fig. 1). Notice that the two-sided confidence limits are separated. Immunization with TAA biological drugs induced strong delayed hypersensitivity reactions which were highly specific; both autologous and allogeneic lung TAA skin testing induced titrated nanogram reactivity. A number of patients returning for their tenth to twelfth anniversary continue to exhibit a strong, specific immunologic memory. Shown in Figure 2 is a comparison of late testing of all patients at 18 months after therapy, showing the continued effect of this form of treatment. This phase II trial was merely an indication of efficacy, and survival data suggested that it would be worthwhile to determine if this experience could be repeated in phase III trials.

Trial 2: Specific Active Immunotherapy Phase III Trial A

We have reported previously a Phase III clinical trial[3] which was conducted in northern New Jersey and northern New York to evaluate therapeutic efficacy of specific active immunotherapy in patients with Stage I (T1N0, T2N0, T1N1 as in phase II trial) and, in addition, Stage II (T2N1M0) squamous cell carcinoma. Pathology slides from participating institutions were reviewed by one pathologist in order to verify diagnoses.

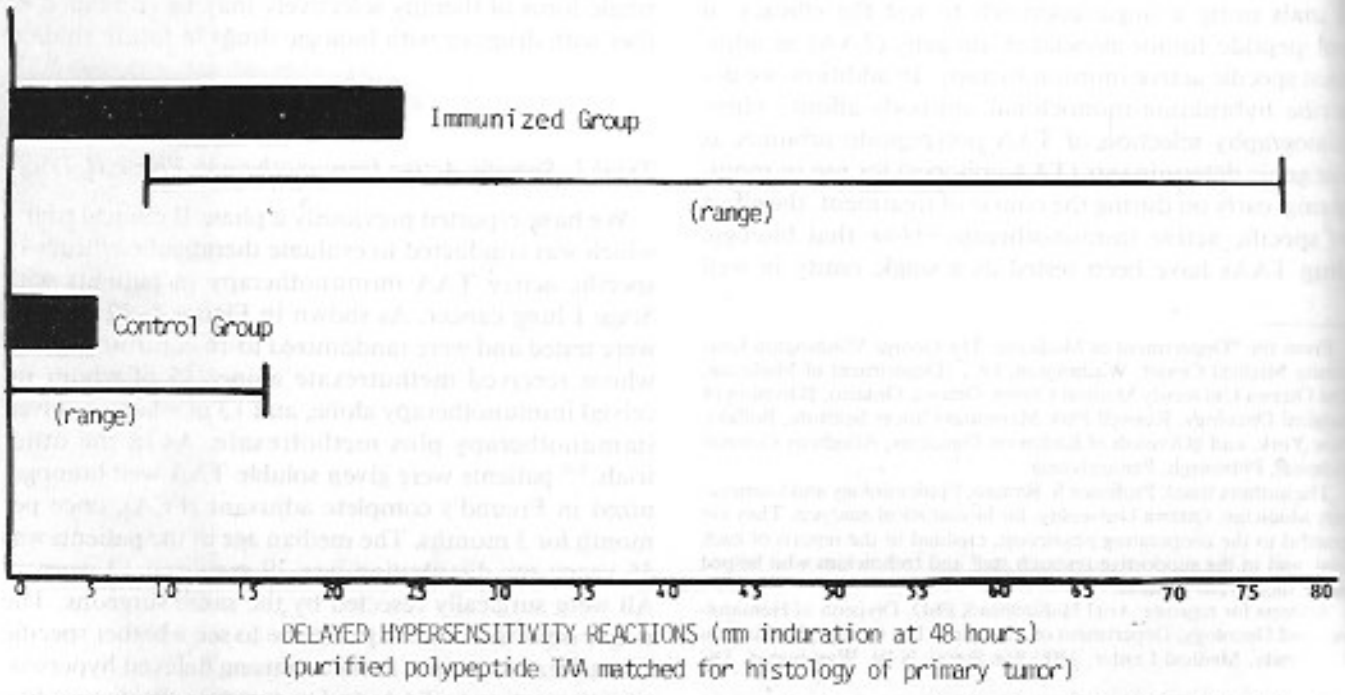


FIG. 2. Long-lasting delayed hypersensitivity reactions in Stage I lung cancer patients as a monitor for the continued effect of specific active immunotherapy.

Within 14 to 21 days after surgery, patients were stratified by stage of disease and randomly allocated by the statistical center of Roswell Park Memorial Institute to one of three experimental groups: I, Control; II, Specific Active Immunotherapy; and III, Adjuvant only arms. As with Trial 1, single doses of TAA at 500 μg per 0.3 to 0.5 ml were kept at −70°C to −80°C, thawed quickly and well emulsified with complete Freund's adjuvant and delivered slowly intradermally to patients on the immunotherapy arm. A total of three immunizations per patient were given, once per month × 3, and no further immunization. Patients on adjuvant arm received 0.2 to 0.5 ml adjuvant emulsified with 0.5 ml normal saline. Delayed hypersensitivity skin tests with 100 μg per 0.1 ml TAA were to have been used to monitor arm II only. Unfortunately, arm III patients were also skin tested at least five times, at months 1, 4, 6, 9 and 12, and, in effect, a degree of TAA specific active immunotherapy was elicited, since the patients had received at least 500 μg TAA. Other investigators have found that delayed hypersensitivity can be induced and strengthened when antigen is given separately but at the same time period of the efficacy of adjuvant.[3] This subgroup, then, was considered as part of the therapy group by the biostatistician for the 10-year analysis. Although this inclusion decreased somewhat the overall survival rates of the therapy arm at the 5-year level, it was adhered to strictly. At the time of our first report of this trial, A, the 3-year survivals[3] looked favorable for this separate-site immunotherapy. We have now arrived at the 5-year level and these patients are indicated by asterisk in the Immunotherapy Group (Table 1) with no significant statistical difference.

The recent 5-year survival curves for this phase III immunotherapy trial A are shown in Figure 3. 66% of Stage I and II lung cancer patients receiving specific active immunotherapy are alive at 5 years as compared with 33% survival in the control group ($P = 0.0056$). The median survival for the treated group is greater than 60 months and the median survival for the control group is 38.5 months.

Trial 3: Specific Active Immunotherapy
Phase III Trial B

A trial similar to Trial 2 was conducted in Stages I and II patients who received TAA of matching histologic type, with cases reviewed for staging and pathologic features and with TAA skin testing confined to the immunotherapy arm except for only one skin test at the 1-year level for the control group and the adjuvant alone group.[4]

After curative surgery for lung cancer, patients with Stage I and Stage II lung cancers were assessed for eligibility and randomized from a third party central office to one of three arms: A, specific active TAA immunotherapy; B, adjuvant alone; and C, control groups. The follow-up was to be 5 years from the date of allocation. The appearance of a definite local recurrence or distant metastasis would permit further therapy at the discretion of the physician with continued follow-up. In this trial, many centers had major protocol violations.[4] One center entered only one patient, a control. Some centers gave no specific active immunotherapy after randomization to that arm. Other centers gave only one immunization. (In our early studies, it was shown that all three injections are necessary in order to effect a long-lasting immune response which peaks at approximately 5 months, plateaus and lasts for many years.) No attempt was made in certain centers to understand how to homogenize adjuvant and TAA despite written and oral instruction. TAA were stored in domestic refrigerators rather than the mandatory −70°C was recognized in two centers, and other incorrect handling errors were identified. However, on site quality surveillance of centers was not possible and attempts to monitor by phone by the principal investigator were unsuccessful.

Nevertheless, the data for all centers, including those with major violations were included in complete detail when negative results of this trial were reported.[4] As shown in Figure 4A, in this 260 patient trial no advantage for any arm was seen. Overall, no bias as to pretreatment characteristics were seen for any arm. The high percentage of protocol violation may invalidate the reliability of the trials results. Although no statistical weight can be attributed to Figure 4B, it can be seen that it is clear that two centers, Ottawa and Pittsburgh, had results which differed. These were the only two centers where the induction of strong delayed hypersensitivity reactions to TAA indicated successful adherence to the protocol rather than the strategic result when major protocol violations were included.[4]

The phase II stage I lung cancer specific active immunotherapy trial was successful, with 5-year survival of 78% in the treatment arm *versus* 46% survival in the control arm. The phase III Stages I and II specific active immunotherapy trial A was successful with 5-year survival of 66% in the treatment arm versus 33% in the control arm. The phase III Stages I and II specific active immunotherapy trial B had many problems and major protocol violations. Here we report on the ten year survival experience to evaluate the efficacy of immunotherapy when the protocol is adhered to strictly, where the induction of strong delayed hypersensitivity responses (DHR) to tumor antigen indicated a successful[1–3] adherence to the protocol rather than the strategic result when major protocol violations were included.[4] All patients, including violations, from centers with acceptable levels are included.

TABLE 1. Specific Active Lung Cancer Immunotherapy: Immunotherapy Group

Patient	Age/sex	Performance status	Type of surgery	Stage	Histologic type	5-yr survival status
1	62M	1	R pneumonectomy	T1N1	WDIF Squam	NED
2	61M	1	LU lobectomy	T2N1	Large cell	NED
3	53F	1	L pneumonectomy	T2N0	Large cell	NED
4	62M	0	LU lobectomy	T1N0	Squam	Died 40 mo
5	61M	1	L pneumonectomy	T2N1	Squam	NED
6	56F	1	RU lobectomy	T2N0	Adeno/Squam	NED
7*	45M	1	R pneumonectomy	T2N0	Adeno	NED
8	66F	1	RU lobectomy	T2N0	Adeno	Died 51 mo
9	64M	0	LL lobectomy	T2N0	Squam	NED
10	45M	1	L pneumonectomy	T2N0	Adeno	NED
11	58M	1	RU lobectomy	T2N0	Large cell	NED
12	52F	1	RU lobectomy	T1N0	Adeno	NED
13	45F	1	L pneumonectomy	T1N1	Large cell	NED
14	59M	1	LU lobectomy	T2N0	Squam	AWD
15	54M	1	L pneumonectomy	T2N0	Large cell	Died 14 mo
16	60M	0	R U&M lobectomy	T2N0	Squam	NED
17	51M	1	LU lobectomy	T2N1	Squam	Died 17 mo
18	57M	1	L pneumonectomy	T1N0	Squam	NED
19	56M	1	L pneumonectomy	T1N0	Squam	Died 42 mo
20	69M	1	L U&L lobectomy	T2N0 T1N0	Squam	Died 55 mo
21	54M	0	LU lobectomy	T2N0	Squam	Died 42 mo
22	71M	0	LU lobectomy	T1N0	Adeno	NED
23	66M	0	LU lobectomy	T1N0	Adeno	NED
24	60M	0	LU lobectomy	T1N0	Squam	NED: Lfu 30 mo
25	40F	1	RM lobectomy	T1N0	Adeno	NED
26	40M	1	LU lobectomy	T2N0	PDIF Squam	NED
27	62M	1	LU lobectomy	T1N1	Squam	NED
28	60M	0	LU lobectomy	T1N0	Oat	Died 27 mo
29	69F	1	RU lobectomy	T2N0	Alveol	NED
30	58M	1	L pneumonectomy	T2N1	WDIF Squam	NED
31	59F	1	RL lobectomy	T1N0	Squam	NED
32*	65M	1	L pneumonectomy	T2N0	WDIF Squam	Died 59 mo
33	60M	0	LL lobectomy	T1N0	Adeno/squam	Died 39 mo
34	66M	1	LL lobectomy	T1N0	MDIF Squam	NED
35*	57M	1	RU lobectomy	T1N0	WDIF Squam	NED
36*	70M	0	LL lobectomy	T2N0	PDIF Squam	NED: Lfu 51 mo
37*	66M	1	RU lobectomy	T2N0	Squam	NED
38*	50M	0	R pneumonectomy	T2N1	MDIF Squam	Died 26 mo
39*	52M	2	L pneumonectomy	T2N1	PDIF Squam	Died 2 mo
40	76F	1	RU lobectomy	T2N0	PDIF Squam	Died 27 mo
41*	66M	1	LL lobectomy	T2N0	PDIF Squam	Died 57 mo
42	59M	1	RL lobectomy	T1N0	Large cell	NED
43	62M	1–2	L pneumonectomy	T2N1	Squam	NED
44	76M	1	LU lobectomy	T2N2	Anap Squam	NED
45	62M	1	LU lobectomy	T1N0	Anap Squam Oat	AWD
46*	44M	1	R pneumonectomy	T2N2	Squam	NED
47*	76M	1	LU lobectomy	T2N0	PDIF Squam	NED
48*	62M	0	R M&L lobectomy	T1N0	MDIF Squam	Died 32 mo
49*	63M	1	R U&M lobectomy	T1N0	Squam	NED
50	63M	2	RL lobectomy	T1N0	Squam	NED
51	70M	0	L pneumonectomy	T2N0	Squam	NED
52	72M	1	RM lobectomy	T1N0	PDIF Squam	NED
53*	69M	1	RL lobectomy	T1N1	Squam	Died 8 mo
54	57M	1	L pneumonectomy	T2N0	MDIF Squam	Died 22 mo
55	71M	1	RU lobectomy	T1N0	MDIF Squam	NED
56*	46M	1	R M&L lobectomy	T2N0	MDIF Squam	NED
57*	60M	1	LL lobectomy	T1N0	WDIF Squam	NED
58	58M	1	RU lobectomy	T2N0	MDIF Squam	Died 21 mo
59	62M	0	LU lobectomy	T1N0	MDIF Squam	NED
60*	48F	1	RU lobectomy	T2N0	Squam	NED
61	61F	1	RU lobectomy	T1N0	Adeno	NED
62	57M	1	RM lobectomy	T1N0	Squam	NED
63*	72M	1	RU lobectomy	T1N0	Squam	Died 42 mo
64*	63M	1	L pneumonectomy	T2N1	MDIF Squam	NED

TABLE 1. (*Continued*)

Patient	Age/sex	Performance status	Type of surgery	Stage	Histologic type	5-yr survival status
65	69F	1	RU lobectomy	T2N0	MDIF Squam	NED
66*	59M	1	LU lobectomy	T1N1	MDIF Squam	NED
67	58M	0	RM lobectomy	T1N0	Squam	NED
68	60M	1	L lobectomy	T2N0	PDIF Squam	NED
69	69M	1	L pneumonectomy	T2N0	MDIF Squam	NED
70*	61M	1	RL lobectomy	T1N1	MDIF Squam	Died 35 mo
71	59M	0	LU lobectomy LL wedge	T2N0	PDIF Squam	Died 47 mo
72*	62M	0	RL lobectomy	T2N1	MDIF Squam	Died 27 mo
73*	58M	0	RL lobectomy	T2N0	MDIF Squam	NED
74*	59M	0	RU lobectomy	T1N0	Squam	AWD
75	65M	0	RU lobectomy	T1N0	MDIF Squam	NED
76	57F	1		T2N0	Large cell	Died 21 mo
77	59M	1	R pneumonectomy	T2N0	Large cell	NED
78	58F	1	LL lobectomy	T1N0	PDIF Squam	NED
79	64M	1	L pneumonectomy	T1N1	Squam	NED
80*	49F	0	L pneumonectomy	T2N1	Squam	NED
81*	57M	0	RU lobectomy	T2N1	PDIF Squam	Died 8 mo
82	51F	0	LL lobectomy	T2N1	PDIF Squam	Died 34 mo
83	60M	1	RU lobectomy	T2N0	Anap Squam	Died 10 mo
84	55M	1	R pneumonectomy	T2N0	Large cell	Died 46 mo
85	52F	1	LL lobectomy	T1N0	MDIF Adeno	NED
86	62M	1	L pneumonectomy	T2N1	WDIF Squam	Died 30 mo
87	62M	1	L pneumonectomy	T2N1	MDIF Squam	NED
88	68M	1	L pneumonectomy	T2N1	MDIF Squam	NED
89	60M	1	L pneumonectomy	T1N0	PDIF Squam	NED
90*	40F	1	R pneumonectomy	T2N1	MDIF Squam	Died
91*	61M	1	L pneumonectomy	T2N1	MDIF Squam	Died 21 mo
92	52F	1	LU lobectomy	T1N1	Adeno	NED
93	51F	0	RU lobectomy	T1N0	Large cell	NED
94	44F	0	RU lobectomy	T2N0	Adeno	NED
95*	68M	0	LU lobectomy	T2N0	MDIF Squam	Died 49 mo
96	62M	1	LU lobectomy	T1N0	Oat	NED
97	51M	1	RL lobectomy	T1N0	MDIF Squam	NED
98	57F	1	RL lobectomy	T2N0	PDIF Squam	Died 8 mo
99	56F	1	L pneumonectomy	T2N0	PDIF Squam	NED
100	47M	1	RL lobectomy	T2N0	PDIF Squam	Died 47 mo
101	53M	1	L pneumonectomy	T2N1	Squam	NED
102	57F	1	LU lobectomy	T2N1	Adeno	NED
103	60F	1	RU lobectomy	T2N0	MDIF Squam	Died 46 mo
104	61M	2	R pneumonectomy	T2N1	MDIF Squam	NED
105	59M	1	LL lobectomy	T2N0	Squam	NED
106	49M	1	R L&M lobectomy	T2N1	Squam	NED
107	67M	1	R pneumonectomy	T2N0	Large cell	NED
108	53M	0	LL lobectomy	T2N0	Adeno	NED: L fu 50 mo
109	56M	1	RL lobectomy	T1N0	Adeno	NED: Lfu 49 mo
110	65M	1	L pneumonectomy	T2N1	PDIF Squam	NED: Lfu 46 mo
111	62M	1	LL lobectomy	T1N1	Large cell	Died 12 mo
112	58M	0	RU lobectomy	T1N0	Adeno	NED: Lfu 43 mo
113	66M	1	RU & RM lobectomy	T1N1	Adeno	Died 18 mo
114	52F	1	RU — RM wedge	T1N1	PDIF Squam	NED: Lfu 42 mo
115	66F	1	LL lobectomy	T1N0	Squam	NED: Lfu 39mo
116	54M	2	LU lobectomy	T1N1	MDIF Squam	NED: Lfu 36 mo
117	66M	1	RU lobectomy	T1N1	Adeno	Died 5 mo
118	62F	2	LL lobectomy	T2N0	PDIF Squam	NED: Lfu 25 mo

* Also included, although received less TAA and separate site FcA. Originally designated as an adjuvant control arm, patients also were skin tested with TAA and therefore this arm could not be considered as an adjuvant control. Whether or not the lesser amounts of TAA used in skin tests, originally to monitor this arm, interfered with adjuvant effects had to be determined by comparing the results of this group with those who received adjuvant FcA without TAA (see control group for adjuvant alone-arm subgroup).

NED: no evidence of disease; AWD: alive with disease; Lfu: last follow-up, not yet at 5 yr, as of March 1986; Alveol: alveolar; RU & RM: right upper and right middle; R: right; L: left; RL: right lower; RU: right upper; LL: left lower; LU: left upper; R M&L: right middle and lower; R U&M: right upper and middle; L M&L: left middle and lower; WDIF: well differentiated; PDIF: partially differentiated; Squam: squamous; Adeno: adenocarcinoma; MDIF: moderately differentiated.

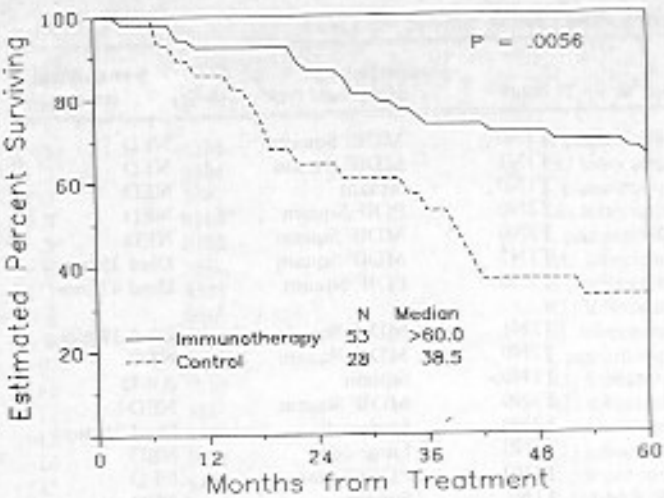
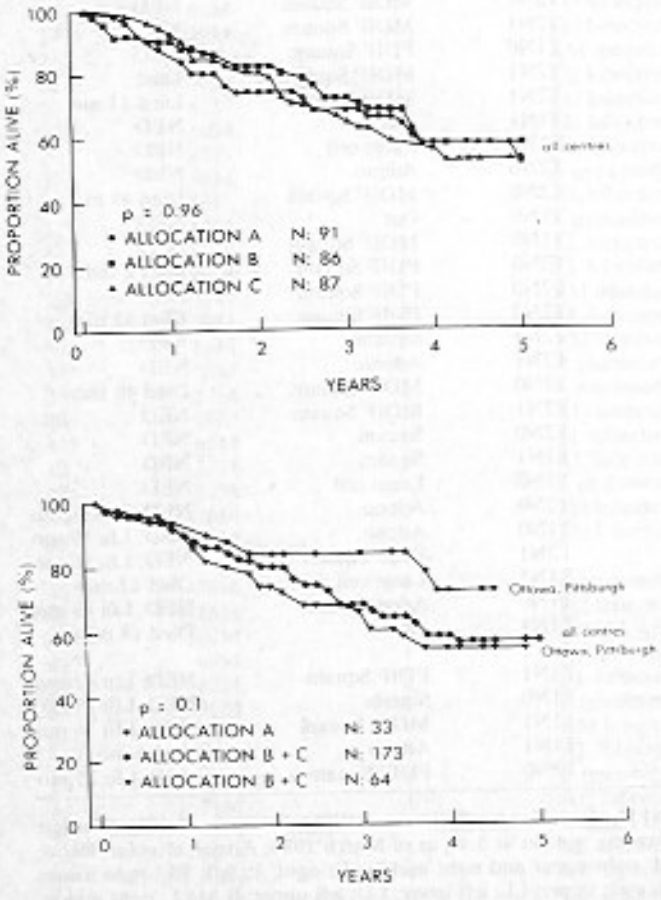


FIG. 3. Phase III specific active immunotherapy trial A in Stages I and II lung cancer patients; 5-year survival.



FIGS. 4A AND 4B. Phase III Specific Active Immunotherapy Trial B in Stages I and II lung cancer patients; 5-year survival. (A, top) Survival: all centers. (B, bottom) Survival: all centers/Ottawa, Pittsburgh. (Figure courtesy of The University of Texas System Cancer Center Annual Clinical Conference on Cancer, vol. 28.)

Materials and Methods

The TAA[1-5] used in this study were purified soluble polypeptides derived from lung cancer cell membranes. The TAA were free of nucleic acid, major human histocompatibility antigens as well as viral and bacterial contaminants. The TAA were identified as lung tumor cell membranes components which produce cell-mediated immunity as measured *in vivo* and *in vitro*. The association with lung primary tumors was established by cross-testing using double immunodiffusion–immunoelectrophoresis, as well as by more sensitive cross-testing using enzyme-linked immunosorbent assays. The TAA have been tested for safety using "Good Laboratory Practices" in accordance with Food and Drug Administration regulations. Aliquots of TAA were prepared from lung tumors having the same histologic type, and the final product was stored in aliquots of 500 μg/0.3 to 0.5 ml for immunizations, and in aliquots of 100 μg/0.1 ml for skin testing. All vials were stored and shipped at less than −70°C, and were stored at −70°C in the cooperating centers. All centers were instructed as to the necessity for complete homogenization with an equal volume of FCA, in that it is necessary to disburse the adjuvant very thoroughly in order to insure that complete rather than incomplete adjuvant capability is established.

Centers which are included in the present evaluation were from northern New York, northern New Jersey, western Pennsylvania and eastern Canada. All groups met together to agree on surgery, pathology, staging and other protocol aspects. It was considered legitimate to pool the data of all patients from the different areas since (1) all trials were randomized, (2) the treatment protocols were the same, (3) the patient exclusion criteria for the trials were the same, (4) the disease categories had not changed over the period of time of the protocol, (5) the patient management regime for the treatment and control groups had not changed, and (6) the same laboratory prepared the vaccines and the principle investigator for one of the trials[4] cross-compared and cross-tested the potency of the vaccines for all groups.

For the purpose of the analysis the follow-up has been limited to 5 years (60 months). Data were analyzed by the life-table techniques using the computer program BMDP PIL (BMDP, Los Angeles, CA). Statistical significance was derived from the Generalized Wilcoxon (GW) or Generalized Savage Test. The results of the tests are usually very close. However, it is known that the GW places a greater weight on early deaths and is less sensitive to late events which occur when few patients are alive. Kaplan-Meier Product density life tables were graphed and the analyses for all cases is very highly statistically significant. The data then were stratified into subgroups to identify categories of interest which

are statistically significant. The results should be interpreted with caution because they are subject to the fallacy of multiple comparisons and smaller sample sizes. Analyses for squamous cell cancer, for each sex, for T1N0, for Stage I, for small tumors and for adjuvant effect show the results as highly significant. Less significant are the results for adenocarcinoma and for Stage II, although within acceptable range for subset analyses reporting, with the above caution on interpretation.

Patients with Stage I and Stage II lung cancers who had curative lung surgery were staged according to the surgical and pathologic findings with the criteria recommended by the American Joint Committee for Cancer Staging and End Results Reporting.[6] After curative surgery for lung cancer, and after signing a detailed consent form, patients were randomized to 1 of 3 arms. The first arm was a control arm that offered customary surveillance but no treatment. A second arm consisted of patients who received adjuvant homogenized in an equal volume of saline, three times over monthly intervals. A third arm consisted of patients who received TAA immunotherapy, which consisted of adjuvant homogenized with TAA, once per month for 3 months total. All immunizations were administered intradermally.

The minor modification[4] was the division of Stage I into two parts, Stage IA and IB. The Stage IA classification included lesions less than 3.0 cm in diameter without involvement of the visceral pleura or invasion of a lobar bronchus (T1). Stage IB included T1 lesions with peribronchial and/or ipsilateral hilar lymph node involvement as well as T2 lesions with negative nodes. A lesion greater than 3 cm in diameter was classified as T2. Lesions involving visceral pleura, atelectasis and/or obstructive pneumonitis limited to the diseased lobe were also included as T2 lesions. In all cases the tumor must be at least 2 cm distal to the corina. A T2 primary lesion with involvement of the peribronchial and/or ipsilateral hilar nodes was staged as Stage II.

Results

As shown in Tables 1 and 2, a total of 234 lung cancer patients were entered,[1–5] and the individual ages, sex, performance status, type of surgery, stage of disease, histologic type, and 5-year survival status are shown in the tables. Not all patients have arrived at the 5-year level, and in this case, the month of last follow-up, as of March 1, 1986, is shown. Also shown is the time of expiration, and the status of patients at the 5-year level, as to whether or not they are alive with disease or show no evidence of disease at the 5-year mark.

The 5-year survival of all cases, Stages I and II, for all 234 lung cancer patients is shown graphically in Figure 5A. The differences between the control group and the immunotherapy group have a P value of 0.0002, and the median, as of this evaluation of March 1, 1986, has not yet been reached for the treatment group. Statistical evaluation at the 95% confidence limits are shown in Figure 5B. The generalized Wilcoxon two-sided analysis of the 95% confidence limits, indicates a significant separation at approximately 22 months and beyond. Results using the generalized Savage test were very close, indicating that the data withstands the test of two separate analyses for statistical significance. Recurrence in the control and treatment groups appeared to be randomly distributed in time. The treatment group appeared to be dying at a much slower rate than the control group. There was no evidence of clustering in either group. Distribution according to stage of disease was as follows: T1N0 controls 47, immunotherapy 37; T2N0 controls 43, immunotherapy 44; T1N1 controls nine, immunotherapy 13; T2N1 controls 17, immunotherapy 22 plus two patients in the immunotherapy group who were T2N2.

In these studies we used TAA prepared from primary lung tumors only. In Figure 6 are shown the survival curves for patients with stages T1N0 and T2N0. These data are most interesting in that the difference in survival observed at 60 months in the smaller clinical trial[1] are repeated in the larger trials.[3–5] The greatest promise for this specific active TAA immunotherapy is seen in patients with small tumors, T1N0, as shown in Figure 7. These differences are highly significant statistically, and point out that the real promise of immunotherapy in an adjunctive treatment setting is with early stage lung cancer.

By contrast, TAA therapy in stage T2N1 cases, Figure 8, is less effective, although a distinct difference is seen at 3.5 years between the patients receiving immunotherapy and those serving as controls. It will be of interest to follow the survival status of these groups. Shown in Figure 9 is the breakout of those patients, both control and therapy groups, with small primary tumors, that is T1N0 and T1N1. Figures 10 and 11 show the survival curves of controls and TAA treated groups for women and for men, respectively. In Table 2, in the control group, the subgroup on a separate arm who received adjuvant only are indicated with an asterisk. It was of importance to see what effect the adjuvant alone might have as a single agent. The next three figures analyze this effect. Shown in Figure 12 is the comparison of survival data for the TAA treated group in Stages I and II lung cancer patients, compared with all controls who did not receive FCA. This can be compared with the survival curves shown in Figure 13 which compares the same treated group with the control group who received adjuvant alone. Figure 14 does not contain the treated group, and illustrates the difference between the controls

TABLE 2. Specific Active Lung Cancer Immunotherapy: Control Group

Patient	Age/sex	Performance status	Type of surgery	Stage	Histologic type	5-yr survival status
1	62M	1	R pneumonectomy	T1N0	MDIF Squam	NED
2	60M	1	R pneumonectomy	T1N1	MDIF Squam	NED
3	73F	0	RL lobectomy	T1N1	Adeno	Died 30 mo
4	45F	1	L pneumonectomy	T1N1	Oat	NED
5	39M	1	LU lobectomy	T2N0	Adeno	NED
6	52M	0	LU lobectomy	T2N0	Oat	Died 46 mo
7	48M	1	LU lobectomy	T2N0	MDIF Squam	NED
8	57F	1	L pneumonectomy	T2N0	Adeno	Died 8 mo
9	54M	0	RU lobectomy	T1N0	Adeno	Died 22 mo
10	60M	1	LU lobectomy	T1N0	MDIF Squam	AWD
11	65M	1	LU lobectomy	T1N0	Squam	NED
12	50M	1	RU lobectomy	T1N0	Adeno	AWD
13	52M	1	RU lobectomy	T2N0	Adeno	NED
14	65M	1	RU lobectomy	T1N0	Squam	Died 58 mo
15	50M	1	R pneumonectomy	T2N0	Large cell	Died 22 mo
16	74M	1	LU lobectomy	T2N0	Adeno	Died 51 mo
17	47F	1	R pneumonectomy	T2N1	Adeno	Died 39 mo
18	60M	1	L pneumonectomy	T2N1	Large cell	Died 7 mo
19	50F	1	LU lobectomy	T2N0	Adeno	Died 27 mo
20	52M	1	LL lobectomy	T2N0	Squam	Died 6 mo
21	45M	1	RM lobectomy	T1N0	Small cell	Died 24 mo
22	64M	1	RU lobectomy	T2N0	Adeno	NED
23	67M	1	LL lobectomy	T2N0	MDIF Squam	NED
24	74M	1	RU lobectomy	T1N0	Alveol	Died 32 mo
25	65F	1	RL lobectomy	T2N0	PDIF Squam	Died 17 mo
26	49F	1	L pneumonectomy	T2N1	ANAPL Squam	Died 8 mo
27	62M	1	LU lobectomy	T1N0	MDIF Squam	Died 6 mo
28	58M	1	R pneumonectomy	T2N0	WDIF Squam	Died 18 mo
29	63M	0	L pneumonectomy	T2N0	MDIF Squam	Died 40 mo
30	60M	1	R pneumonectomy	T2N0	WDIF Squam	NED: Lfu 30 mo
31	67M	1	LU lobectomy	T1N0	PDIF Squam	Died 35 mo
32	73M	1	R pneumonectomy	T1N0	MDIF Squam	Died 52 mo
33	70M	1	RL lobectomy	T1N0	PDIF Squam	Died 14 mo
34	63M	1	LU lobectomy	T1N0	Squam	AWD
35	74M	1	LL lobectomy	T2N0	MDIF Squam	Died 18 mo
36	71M	1	R pneumonectomy	T2N0	MDIF Squam	Died 38 mo
37	54M	0	L pneumonectomy	T2N0	MDIF Squam	Died 26 mo
38	67M	1	R M&L lobectomy	T2N0	WDIF Squam	Died 6 mo
39	61M	0	LL lobectomy	T2N1	Squam	NED
40	79M	1	L pneumonectomy	T1N0	MDIF Squam	Died 39 mo
41	63M	0	L pneumonectomy	T2N1	PDIF Squam	NED
42	74F	1	RU lobectomy	T2N0	MDIF Squam	Died 33 mo
43	67M	1	RL lobectomy	T1N0	PDIF Squam	Died 17 mo
44	69M	0	LU lobectomy	T1N0	WDIF Squam	Died 41 mo
45	66M	1	R pneumonectomy	T2N0	Squam	NED
46	69M	1	RU lobectomy	T1N0	MDIF Squam	NED: Lfu 31 mo
47	60F	1	L pneumonectomy	T2N1	MDIF Squam	Died 21 mo
48	57M	1	R M&L lobectomy	T2N0	MDIF Squam	Died 16 mo
49*	56M	1	RU lobectomy	T2N1	PDIF Squam	Died 44 mo
50	55F	1	RL lobectomy	T1N0	Adeno	NED
51	71M	0	RU lobectomy	T2N0	MDIF Squam	NED
52*	65F	1	LL wedge	T1N0	WDIF Adeno	AWD
53*	58F	0	Pneumonectomy	T2N0	PDIF Squam	Died 23 mo
54	54F	1	LL lobectomy	T2N0	MDIF Squam	NED
55	59M	1	RU lobectomy	T1N0	PDIF Squam	Died 24 mo
56	64M	1	LU lobectomy	T2N0	Large cell	Died 43 mo
57*	62M	1	RU lobectomy	T2N0	Adeno	Died 24 mo
58*	68M	1	LU lobectomy	T2N0	Large cell	NED
59	61M	1	LU lobectomy	T1N0	Squam	NED
60*	58M	1	RU lobectomy	T1N0	PDIF Squam	Died 38 mo
61	68M	1	LU lobectomy	T1N0	Squam	Died 15 mo
62	50M	1	LL lobectomy	T1N0	Adeno	NED
63	64M	1	LU lobectomy	T1N1	PDIF Squam	AWD
64*	60M	1	LU lobectomy	T1N0	MDIF Squam	NED
65	70M	1	LU lobectomy	T1N0	Squam	NED
66*	63M	1	L pneumonectomy	T2N1	PDIF Squam	Died 10 mo

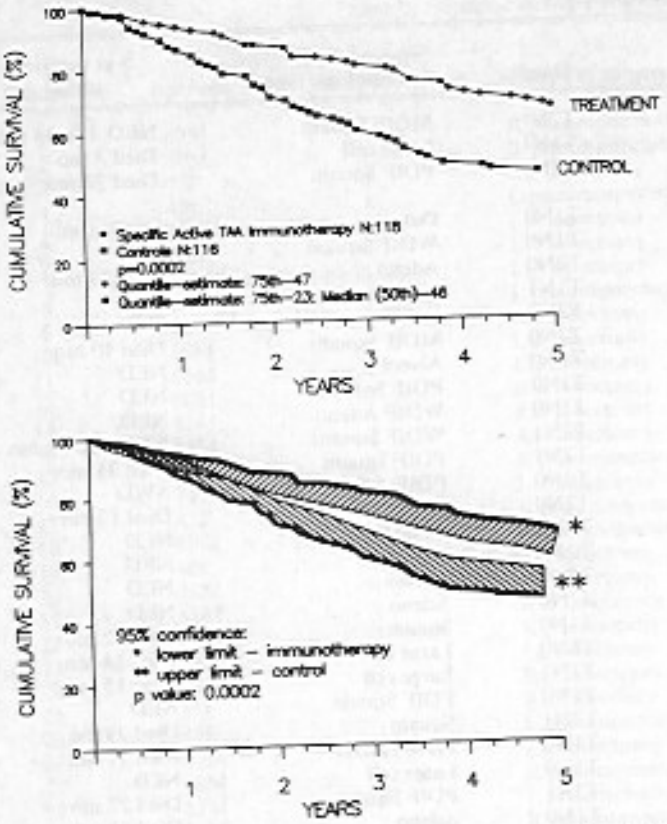
TABLE 2. *(Continued)*

Patient	Age/sex	Performance status	Type of surgery	Stage	Histologic type	5-yr survival status
67	71F	1	L pneumonectomy	T2N1	MDIF Squam	NED: Lfu 34 mo
68*	53M	1	LL lobectomy	T1N1	Large cell	Died 3 mo
69*	70M	1	R U&M Lobectomy	T2N1	PDIF Squam	Died 22 mo
70	59M	1	LU lobectomy	T1N0	Oat	Died 11 mo
71	58M	1	LU lobectomy	T1N0	WDIF Squam	NED
72	63F	1	LU lobectomy	T2N0	Adeno	Died 27 mo
73*	54M	1	L pneumonectomy	T1N1	Adeno	NED
74*	46M	1	L pneumonectomy	T2N0	WDIF Squam	NED
75	63M	0	R U&M lobectomy	T2N0	MDIF Squam	Died 10 mo
76	48F	1	RL lobectomy	T1N0	Alveol	NED
77*	66M	0	RU lobectomy	T1N0	PDIF Squam	NED
78*	62M	0	RM lobectomy	T1N0	WDIF Adeno	NED
79	71M	1:2	RM lobectomy	T2N1	WDIF Squam	NED: Lfu 32 mo
80	66M	1	RL lobectomy	T2N1	PDIF Squam	Died 35 mo
81	50M	1	L pneumonectomy	T2N0	PDIF Adeno/Squam	AWD
82	55M	1	RL lobectomy	T2N0	Alveol	Died 12 mo
83*	51M	1	LL lobectomy	T1N0	PDIF Squam	NED
84	65M	1	RU lobectomy	T2N0	Squam	NED
85*	41F	1	LL lobectomy	T2N1	Adeno	NED
86*	63M	1	RU lobectomy	T1N0	Adeno	NED
87*	50M	1	RL lobectomy	T1N0	Squam	Died 12 mo
88	52F	1	RL lobectomy	T2N0	Large cell	Died 14 mo
89	66M	1	RU lobectomy	T1N1	Large cell	Died 15 mo
90*	57M	1	RU lobectomy	T1N0	PDIF Squam	NED
91	70M	1	LU lobectomy	T2N1	Squam	Died 29 mo
92*	62M	1	RU lobectomy	T1N0	PDIF Adeno	Died 11 mo
93	67M	1	RU lobectomy	T1N0	Large cell	NED
94	58M	1	LL lobectomy	T2N1	PDIF Squam	Died 27 mo
95*	67F	1	RU lobectomy	T1N0	Adeno	Died 41 mo
96	60M	1	LL lobectomy	T1N0	WDIF Squam	NED: Lfu 55 mo
97*	54M	0	RU lobectomy	T1N0	Large cell	Died 34 mo
98	50F	1	LU lobectomy	T1N0	Large cell	NED: Lfu 49 mo
99*	47M	0	LU lobectomy	T2N0	Large cell	NED: Lfu 50 mo
100*	58F	1	LU lobectomy	T1N0	Adeno	NED: Lfu 45 mo
101*	57M	1	L pneumonectomy	T2N1	PDIF Squam	NED: Lfu 44 mo
102*	54F	1	L M&L lobectomy	T2N0	Oat	Died 43 mo
103	59M	0	RU lobectomy	T1N0	Large cell	NED: Lfu 47 mo
104	59M	1	RU lobectomy	T1N0	Squam	Died 23 mo
105	59F	2	L pneumonectomy	T2N0	MDIF Squam	NED: Lfu 44 mo
106	55F	1	RU lobectomy	T1N0	Large cell	NED: Lfu 43 mo
107	55M	1	LU lobectomy	T1N1	PDIF Squam	NED: Lfu 37 mo
108*	51M	1	RU lobectomy	T2N0	PDIF Adeno	NED: Lfu 45 mo
109*	61M	2	LL lobectomy	T2N0	Large cell	Died 5 mo
110*	68M	1	RU lobectomy	T2N0	Adeno	NED: Lfu 39 mo
111*	60M	1	RU lobectomy	T2N1	WDIF Adeno	AWD
112	68M	1	RU lobectomy	T2N0	Squam	Died 1 mo
113	61M	1	LL lobectomy	T1N0	MDIF Squam	NED: Lfu 36 mo
114	69M	1	L pneumonectomy	T2N1	Adeno	NED: Lfu 36 mo
115	54F	1	RU lobectomy	T1N0	Adeno	Died 28 mo
116*	70M	1	LU lobectomy	T2N0	MDIF Adeno	NED: Lfu 26 mo

* Also received adjuvant. Adjuvant arm survival rate was same as controls, so two groups are placed together as total control group.

NED: no evidence of disease; AWD: alive with disease; Lfu: last follow-up, not yet at 5 yr, as of March 1986; R: right; L: left; RL: right lower; RU: right upper; LL: left lower; LU: left upper; R M&L: right middle and lower; R U&M: right upper and middle; L M&L: left middle and lower; WDIF: well differentiated; PDIF: partially differentiated; Squam: squamous; Adeno: adenocarcinoma; MDIF: moderately differentiated.

who did or did not receive adjuvant. A slight advantage in survival was seen in patients receiving adjuvant; however, the difference was not statistically significant and indicates that the FCA only and untreated control groups may be combined for statistical analysis. It is useful to note this slight advantage, although not significant, in that this would indicate that the use of the FCA adjuvant is doing no harm, and we note the usefulness of very tiny amounts of this classical adjuvant, and the granulomatous formation at the intradermal site, con-



FIGS. 5A AND 5B. Ten-year survival experience evaluations. Total survival experience in 234 lung cancer Stage I and II patients. (A, top) Survival, all cases. (B, bottom) Specific active immunotherapy (all cases: 95% confidence limit; two-sided analysis. Test statistics: (GW) Generalized Wilcoxon (Breslow), statistic = 13.763, DF = 1, *P* value = 0.0002; (GS) Generalized Savage (Mantel-Cox), statistic = 12.854, DF = 1, *P* value = 0.0003. Pattern of deaths among control and treatment groups appear to be randomly distributed in time. The treatment group appears to be dying slower than the controls. There was no evidence of clustering in either group.

sisting of a large number of macrophages. Although other forms of adjuvant have been tried,[7] they have not proven useful. Until better adjuvant are available it appears that FCA is the adjuvant of choice at this time. Similarly, a subcutaneous route of administration has proven to be ineffective,[7] and optimum therapeutic efficacy is only realized with intradermal immunization. Since the major forms of lung cancer in this trial consist of patients with adenocarcinoma or squamous cell cancer, survival differences in these 2 major histopathologic types of cancer were assessed. Survival curves for patients with Stages I and II epidermoid bronchogenic carcinomas are shown in Figure 15. A statistically significant difference was observed between patients treated with epidermoid TAA and the control group. In Figure 16, survival of all adenocarcinoma cases are compared; for the first 2 years the survival experience is about the same, with a sharp difference and advantage seen in the next 2 years for patients who received adenocarcinoma specific active TAA immunotherapy. A final evaluation of these data will not be conducted until all patients have reached their 5-year anniversary. However, this interim analysis as of March 1, 1986, is statistically valid and indicates the therapeutic advantage accruing to patients undergoing specific active TAA immunotherapy.

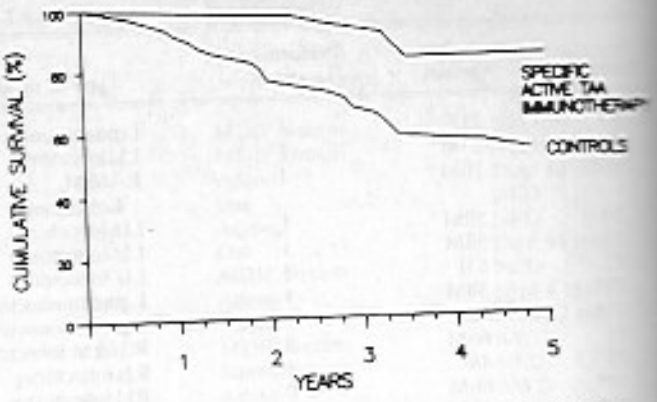


FIG. 7. Ten-year survival experience evaluations. Survival: Stage I with small tumors: T1N0. GW *P* value: 0.0023; GS *P* value: 0.0033.

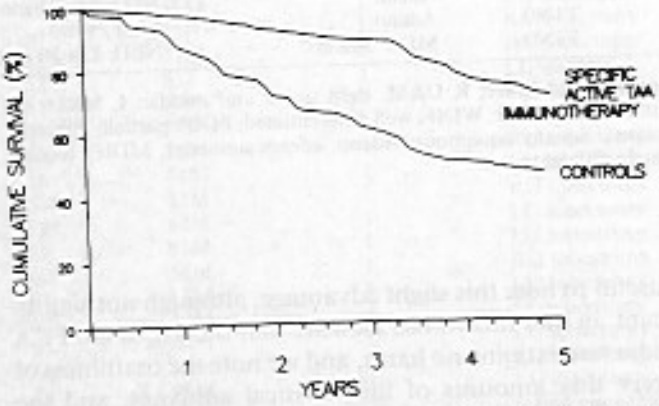


FIG. 6. Ten-year survival experience evaluations. Survival: T1N0, T2N0. GW *P* value: 0.0001; GS *P* value: 0.0003.

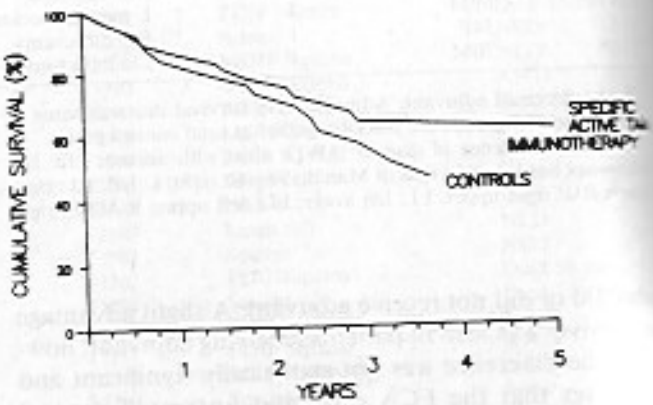


FIG. 8. Ten-year survival experience evaluations. Survival: all Stage II cases. GW *P* value: 0.3985. GS *P* value: 0.3142.

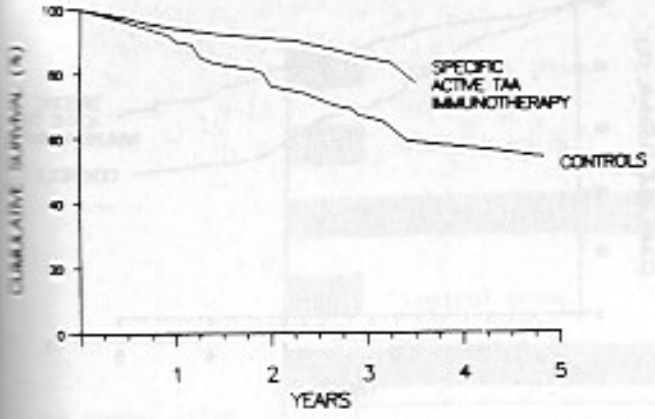


FIG. 9. Ten-year survival experience evaluations. Survival: all small tumors, T1N0, T1N1. GW *P* value: 0.0168; GS *P* value: 0.0157.

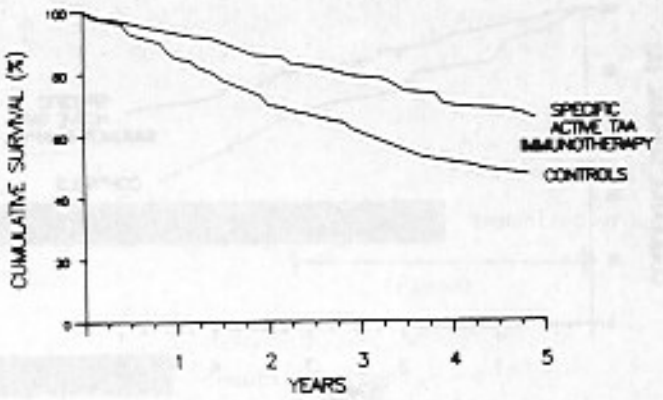


FIG. 11. Ten-year survival experience evaluations. Survival: all males. GW *P* value: 0.0054; GS *P* value: 0.0075.

Serial monitoring techniques were used for the evaluation of patients on these trials to measure various immune responses both prospectively and retrospectively. These studies are useful for the design of future trials, which may involve the combination of this form of treatment with other promising therapeutic agents, including drug therapy and biologic drug therapy. One of these monitoring techniques has permitted us to monitor the patient's early response to immunotherapy and to predict early on in the course of the trial whether or not that patient has responded to immunization. In a series of experiments to develop the assays, we used highly purified antigen to prepare monoclonal antibody-derived epitopes (active peptides of TAA protein).[8] Certain monoclonal antibody-derived epitopes were more sensitive and selective for detection of antibodies in patient sera than were monoclonal antibodies alone used in competitive indirect enzyme immunoassays. Some of the lung tumor TAA epitopes are nonspecific, while others appear to be quite specific with regard to well-defined primary lung tumor histopathologic subtypes. Shown in Figure 17 is an example of enzyme assays performed to epitope D36h6. In this study, monoclonal antibodies were prepared to a lung squamous cell TAA which in purified form is 37,000 daltons. As illustrated in Figure 17, monoclonal antibody was coated on the beads of an affinity chromatography column, and highly purified squamous cell TAA was poured over the column and the resulting antigen–antibody complexes eluted from the affinity column were separated in order to derive the immunoreactive peptides which were studied for activity (epitopes) and for cross-reactions.[9] After comparing several methods, we selected the enzyme immunoassay as the best way to test these epitopes.[10] D36h6 was selected as the least cross-reactive of the monoclonal antibody purgation derived epitopes prepared from the 37Kd TAA. Only 5% of nonlung cancer and nonsquamous cell cancer sera reacted in the assay, and then only at upper concentra-

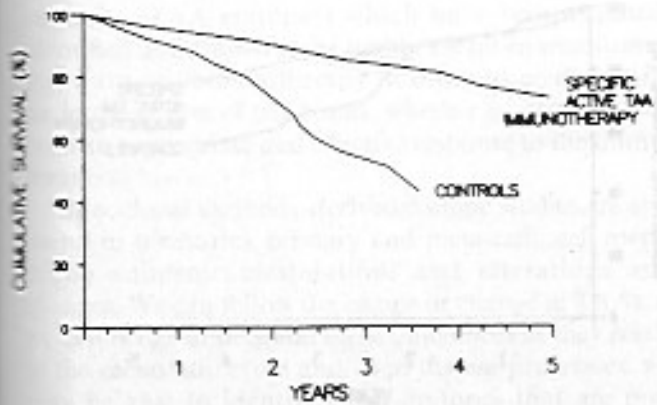


FIG. 10. Ten-year survival experience evaluations. Survival: all females. GW *P* value: 0.0073; GS *P* value: 0.0092.

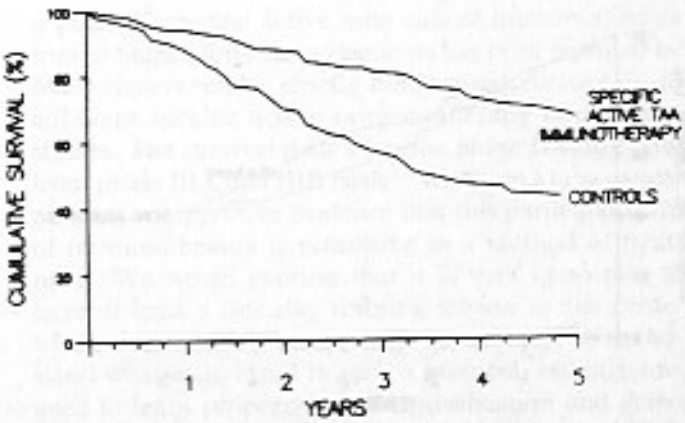


FIG. 12. Ten-year survival experience evaluations. Survival: control group consists of those not receiving adjuvant. GW *P* value: 0.0001; GS *P* value: 0.0001.

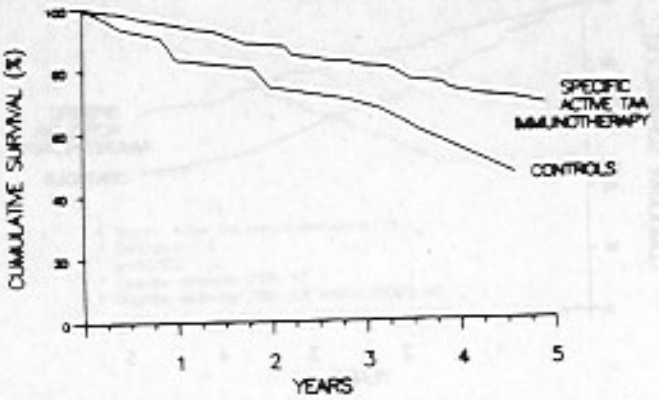


FIG. 13. Ten-year survival experience evaluations. Survival: control group consists of those receiving adjuvant. GW *P* value: 0.0877; GS *P* value: 0.1157.

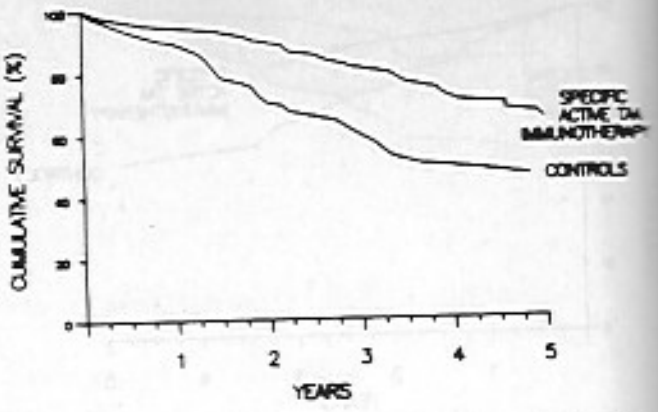


FIG. 15. Ten-year survival experience evaluations. Survival: all squamous cell cancer cases. GW *P* value: 0.0033; GS *P* value: 0.0071.

tions greater than 60 ng which was outside the limits of test sensitivity. There were no cross-reactivities seen to normal sera. Thus, any control sera responses were at the limits of the reaction and did not compare to levels of sensitivity seen in sera from patients at indicated time periods after receiving squamous TAA specific active immunotherapy. All sera were from nonanergic, untreated patients and from age–sex matched controls. In one set of immunoassay studies, Figure 17, dilutions of the epitope, ten-fold, 60 to 10 ng, were tested in triplicate against five (first, second, third monthly immunization periods, at 5–6 months and at 9–12 months) serial sera from each of 22 patients who had received immunotherapy, and from each of 20 matched patient controls. This was a total of 3780 enzyme-linked immunosorbent assays. There is (Figure 17), a burst of antibody to the epitope at or shortly after the second immunization. This level increases at the third immunization and remains or keeps rising for another 2 to 3 months and then subsides (to the tissues). In contrast, in a separate study of patients with Stage III who failed therapy, there was no rise in antibody response seen at the time of the first and second immunization. One patient showed a positive response at the weakest limit at the third immunization, and all patients who failed therapy also failed to sustain antibody responses, as measured by negative responses at 6 months. This particular type of monitoring may permit us to measure specific responses to TAA immunization, and may be useful and sensitive enough for early monitoring so as to provide prognostic signals as to the efficacy and/or futility for continuing specific active immunotherapy. Thus, we have the capability to monitor early antibody rises in the blood stream and, see Figure 2, a way to monitor the continued effect of TAA immunotherapy and the long-lasting cell related and cell-mediated delayed hypersensitivity reactions.[1,2,4,8] Further reports are planned when all patients have arrived at the 5-year mark.

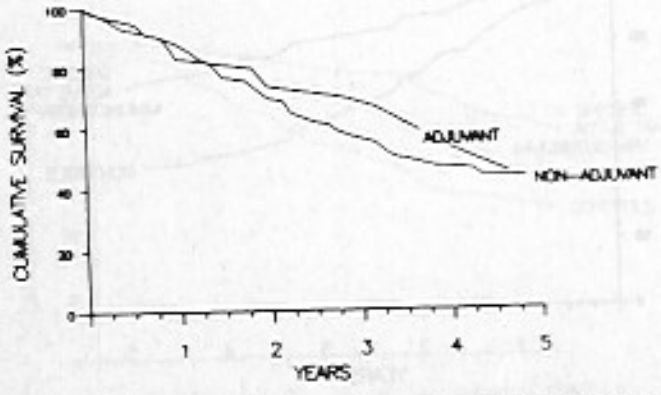


FIG. 14. Ten-year survival experience evaluations. Survival: comparison among controls receiving adjuvant with controls not receiving adjuvant. GW *P* value: 0.3336; GS *P* value: 0.2770.

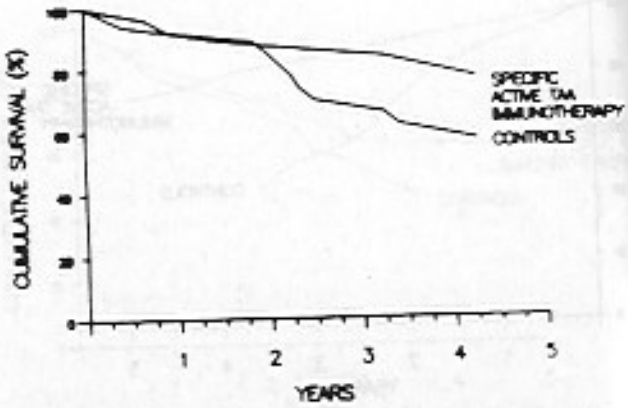


FIG. 16. Ten-year survival experience evaluations. Survival: all adenocarcinoma. GW *P* value: 0.1761; GS *P* value: 0.1604.

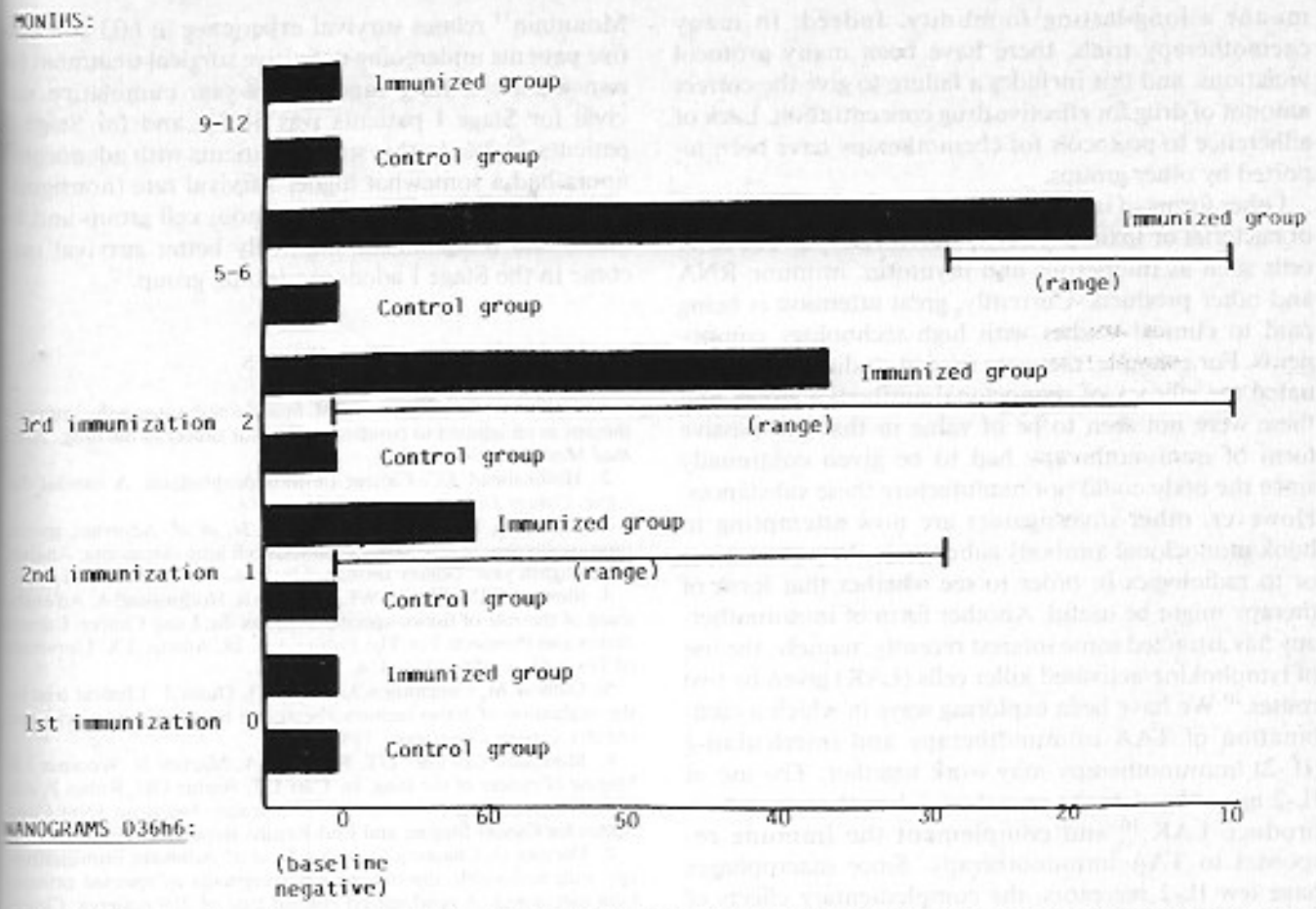


FIG. 17. Monitoring for early response to specific active immunotherapy by monoclonal antibody-derived TAA epitope enzymeimmunoassays all nonsquamous (special pathology assessments) immunized patients and matched control nonimmunized lung cancer patients reacted below the 60 ng level, the limits of test sensitivity; this control group is compared with immunized cancer patients) (baseline negative is >60 ng epitope D36h6).

Discussion

In this report, we describe results in lung cancer treatment using separated polypeptides, lung TAA, in specific active immunotherapy trials, and the use of hybridoma-monoclonal antibody engineered polypeptide subunits (TAA epitopes) which have been isolated, identified and shown to be highly useful in monitoring this form of immunotherapy in order to predict, early on in the course of treatment, whether or not there has been an appropriate and effective response to the immunization.

Monoclonal antibody-derived epitope studies are also useful in measuring primary and metastatic cell membrane antigenic modulations and alterations and changes. We can follow the escape or change in TAAs. If we can better understand these differences as they relate to the metastatic event and/or as disease progresses, we may be able to identify other epitopes that are programmed to support tumor escape mechanisms. In addition, a clearer understanding of surface changes may permit the development of genetically engineered polyvalent TAA preparations for treatment of later stages of lung cancer, in combination strategies with other forms of treatment.

We believe that this is the first time that the results of a phase II specific active lung cancer immunotherapy trial in Stage I lung cancer patients has been repeated by other centers, under strictly randomized conditions, in adjuvant specific active immunotherapy multicenter studies. The survival data from the phase II study[1] and from phase IIIA and IIIB trials[3-5] for Stage I lung cancer patients is supportive evidence that this particular form of immunotherapy is promising as a method of treatment. We would caution that it is very important to have at least a one day training session in the center where this form of therapy is given, in order to understand what is involved in such a protocol. Investigators need to learn proper vaccine emulsification and delivery, and to realize that it is important to follow through with consecutive monthly immunizations, since all three monthly immunizations are necessary in order to

mount a long-lasting immunity. Indeed, in many chemotherapy trials, there have been many protocol violations, and this includes a failure to give the correct amount of drug for effective drug concentration. Lack of adherence to protocols for chemotherapy have been reported by other groups.

Other forms of immunotherapy have included the use of bacterial or toxin products, intermediary products of cells such as interferons and thymosin, immune RNA and other products. Currently, great attention is being paid to clinical studies with high technology components. For example, there are several studies which evaluated the efficacy of monoclonal antibodies alone, and these were not seen to be of value in that this passive form of immunotherapy had to be given continually since the body could not manufacture these substances. However, other investigators are now attempting to hook monoclonal antibody subunits to drugs, to toxins, or to radiologics in order to see whether that form of therapy might be useful. Another form of immunotherapy has attracted some interest recently, namely, the use of lymphokine-activated killer cells (LAK) given by two routes.[10] We have been exploring ways in which a combination of TAA immunotherapy and interleukin-2 (IL-2) immunotherapy may work together. The use of IL-2 may stimulate the growth of T-lymphocytes which produce LAK,[10] and complement the immune responses to TAA immunotherapy. Since macrophages have few IL-2 receptors, the complementary effects of TAA on this pathway as well as on other pathways may be important. TAA immunotherapy gives rise to a long-lasting, cell-mediated immune response, and the use of IL-2 may increase the number of activated T-cells and shoal up the attack upon the tumor using this pathway. The exciting prospect of increasing the efficacy of specific active immunotherapy, particularly in early stage lung cancer, by combining these two biologic drugs may be an approach of promise.

It is of interest to compare our multicenter study survival rates in the control groups with rates reported from another study from a single center. A recent report from Mountain[11] relates survival experience in 603 consecutive patients undergoing definitive surgical treatment for non-small cell lung cancer. Five-year cumulative survival for Stage I patients was 58.2% and for Stage II patients 33.2%. In this study[11] patients with adenocarcinoma had a somewhat higher survival rate (nonsignificant statistically) than the squamous cell group and females had a significant markedly better survival outcome in the Stage I adenocarcinoma group.

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