META ANALYSIS OF CLINICAL TRIALS OF SPECIFIC IMMUNOTHERAPY OF NON SMALL CELL LUNG CANCER

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This paper is a survival analysis of stage I and II lung cancer patients enroled in three clinical trials of adjuvant specific active tumour associated antigen (TAA) immunotherapy. The meta analysis is restricted to completely immunized patients who received the full course of immunization in comparison with the corresponding non immunized control group. Consistent with the theme of this workshop we have also examined the delayed hypersensitivity reaction (DHR) at one year following immunization in relation to tumour dormancy. Five year survival experience of the immunized patients and the corresponding controls is reported.

I. METHODS AND MATERIALS

The lung cancer cases included in this study were all from previously reported clinical trials.[1,2,3]

A. PHASE II TRIAL (1972-76)[1]

A total of 41 stages I and II lung cancer patients from Eastern Ontario and Western Quebec were randomly allocated to three treatment arms: 17 patients received intradermally soluble TAA well homogenized in Freund's complete adjuvant (FcA) once per month for 3 months; 14 patients received methotrexate in addition to TAA immunotherapy and 10 patients received methotrexate alone. It was subsequently found from the records there were 16 patients who were eligible to be included in the trial but were missed. These 16 non-randomized patients were included with the controls.

B. ROSWELL PARK TRIAL (1978-82)[2]

This study consisted of a total of 81 lung cancer patients from the Northern New Jersey and New York area with stages I and II squamous cell carcinoma, who were randomly allocated to three treatment arms: 25 patients received the aforementioned TAA immunotherapy; 28 patients received standard control treatment and 28 patients received vaccination with FcA only. Unfortunately, arm III patients were repeatedly skin tested at least five times during the first year with TAA resulting in the development of a partial immunity and hence this group was merged with the immunized group for the survival analysis.

C. NATIONAL CANCER INSTITUTE OF CANADA (NCIC) TRIAL (1978-83)[3]

This trial included a total of 264 stages I and II lung cancer patients from 10 centres in Canada and 2 centres in U.S., randomly

allocated to three treatment arms: 91 patients received TAA immunotherapy as above; 86 patients received FcA only and 87 patients received standard control treatment. The randomization was stratified by participating centres so that patient allocation was locally balanced and hence each centre could be analyzed separately. Among the Canadian centres all but two centres (Ottawa and Halifax) entered less than ten patients per arm. Further, among nine out of the ten centres (except Ottawa) there were serious protocol deviations such as failure to administer all three immunizations, using incomplete Freund's adjuvant, not refrigerating the vaccines properly; which have been reported previously[3]. Hence, for the purpose of the survival analysis only three centres with minimal protocol deviations (Ottawa, Pittsburgh and Chicago) comprising 47 TAA immunized, 43 FcA controls and 45 standard controls have been included. Further, it was found that in Chicago, though there were no serious protocol deviations, for some unknown reason the vaccinated patients failed to have adequate skin hypersensitivity reaction upon comparison with Ottawa or Pittsburgh, which is indicative of unsuccessful immunization. However the meta analysis includes the data for Chicago though the immunized patients at Chicago could not be considered effectively immunized as measured by the induction of strong DHR (≥20 mm) at one year.

The details of the preparation of the vaccine have been described elsewhere (This volume, Hollinshead). Vaccine used for the Phase II trial was derived from small lots of allogeneic tumors. For the Roswell Park and NCIC trials, the vaccines were prepared in a single uniform batch from a larger batch of allogeneic tumour specimens.

Mortality information was derived directly by contacting the patient or his immediate relative. For patients lost to follow up the known survival status at the time of last contact was used.

1. Statistical Analysis

Life table analysis using the computer program BMDP1L[4] was used to derive estimates of Kaplan-Meier product density survival curves and the statistical significance between the survival curves were calculated using the Mantel-Cox Chi Square[5].

Cox's proportional hazards regression model was used to estimate the effect of immunization. The model is:

$$h(t) = h_0(t)e^{B.X}$$

where h(t) is the hazard rate (the probability of instantaneous death for a survivor at time t), X is an indicator variable for the immunization status (i.e., X = i for the immunized and 0 for the controls) and B is the corresponding regression coefficient. It follows that the hazard ratio, or the relative risk of mortality for a survivor at time t, for an immunized person compared to a non immunized control is e^B. Confounding variables were adjusted using a stratified analysis.

2. Criteria for Pooling the Data for the Meta Analysis

Since the data were collected from three different clinical trials there must be a common basis for combining the data for analysis. The following criteria were used for pooling the data to increase the sample size for the statistical analysis.

1. Patients should be randomly allocated to the immunized and control groups.
2. The treatment protocols used must be the same.
3. The patient inclusion/exclusion criteria for the trials must be similar.
4. Patient management regime for the control and immunized groups must be similar.
5. Patient recruitment occurred during approximately the same time periods.
6. The vaccine used in the trials must be similar.

Effective immunization was defined as the induction of strong DHR (≥20mm) to tumour antigen at one year following immunization. Using this criteria it is apparent that the Chicago data cannot be used for calculating the efficacy of vaccination. In this meta analysis we have shown results of survial analysis including Chicago data. However, we have excluded this data while evaluating the efficacy of the immunization on survial. The Roswell Park Trial data cannot be used to evaluate DHR since the trial did not report DHR at one year. Hence only the NCIC data for Ottawa and Pittsburgh and the Phase II trial data can be used for evaluating the influence of the immunization as measured by the induction of strong DHR at one year.

The Phase II trial data though it violates criteria 1 and 5 was included in the analysis to increase the sample size for statistical power. Concurrent analysis was also done (see tables) excluding Phase II data to assess the robustness of the results.

3. Statistical Power Calculations

The combined sample sizes are adequate to detect a 50% increase in survival time (or a hazard ratio of 0.67) among the immunized at an alpha level of 5% (two-sided) and a power of 90%[6]. However the stated power is attained only after all the patients are followed up until death. To analyze the data during the intermediate stage the power must be recomputed. On the basis of the observed number of deaths among the two groups so far, it was calculated that a 100% decrease (halving) of the mortality risk (hazard ratio of 0.5) among the immunized can be detected at an alpha level of 5% (two-sided) with a statistical power of 91%.

II. RESULTS

A total of 272 subjects were included in this meta analysis of which 131 were immunized and 141 were controls. The controls consist of a combination of patients given standard treatment and those receiving FcA alone, since it has been shown that non specific therapy with FcA alone did not significantly change survival. There were a total of 108 deaths; 43 among immunized and 65 among controls during a five year follow up

period.

TABLE 1
Distribution of charateristics and potentially confounding variables for the Immunized and Control groups.

Variable	Immunized		Control	
	%	n	%	n
Age:(years)				
<50	9.9	13	7.1	10
50-59	32.8	43	29.8	42
60-69	39.7	52	34.8	49
>70	6.9	9	11.3	16
Not stated	10.7	14	17.0	24
Sex:				
Male	75.6	99	75.2	106
Female	24.4	32	24.8	35
Histology:				
Squamous	68.7	90	51.1	72
Adeno	17.6	23	32.6	46
Other	13.7	18	16.3	23
Stage:				
T1N0	32.8	43	43.3	61
T1N1	10.7	14	7.1	10
T2N0	36.6	48	35.5	50
Stage I	(80.2)	(105)	(85.9)	(121)
T2N1	18.3	24	14.2	20
T2N2	1.5	2	0	0
City:				
Ottawa	40.5	53	46.8	66
Buffalo	40.5	53	19.9	28
Pittsburgh	8.4	11	16.3	23
Chicago	10.7	14	17.0	24

Table 1 shows the distribution of the characteristics among the immunized and control groups including the potentially confounding variables. The distribution of the characteristics among the immunized and control are within chance variations except for histology and city (P=.007 and .001 respectively). These imbalances arose due to the fact that the Roswell Park Trial consisted entirely of squamous cell carcinoma and due in the same trial to the merger of the 28 skin tested patients (arm II) with the immunized group. Also, though not statistically significant, the immunized, in respect of prognostic factors influencing lung cancer mortality such as sex and stage, have an unfavourable distribution.

TABLE 2

Distribution of deaths and Five Year Survival Rates for the Immunized and Control patients by categories of potentially confounding variables

Variable	Immunized		Control		
	No. dead/ No.at risk	5 year (S.E) Survival rate	No. dead/ No.at risk	5 year (S.E) Survival rate	P value
Overall	43/131	65.9(4.2)	65/141	51.9(4.4)	0.01
All randomized[a]	28/ 86	65.8(5.3)	45/ 91	48.2(5.5)	0.002
Effectively Immunized[b]	37/117	67.0(4.5)	59/117	47.0(4.8)	0.0007
Sex:					
Male	30/ 90	65.5(5.1)	45/ 91	47.9(5.5)	0.005
Female	7/ 27	72.2(9.0)	14/ 26	45.3(9.9)	0.007
Age:					
≤60	19/ 65	69.4(5.9)	28/ 60	52.4(6.6)	0.02
>60	18/ 52	64.0(6.8)	31/ 57	41.3(7.0)	0.03
Stage:					
T1N0	6/ 36	82.7(6.5)	21/ 48	54.4(7.5)	
T1N1	5/ 13	61.5(13.5)	3/ 8	62.5(17.1)	
T2N0	16/ 44	61.5(7.6)	25/ 43	38.8(7.9)	
Stage 1	27/ 93	69.3(5.0)	49/ 99	48.4(5.2)	0.000
T2N1 & T2N2	10/ 24	58.0(10.1)	10/ 18	38.8(12.7)	
Histology:					
Squamous	30/ 90	65.0(5.2)	37/ 72	45.9(6.1)	0.006
Adeno	6/ 23	72.1(9.8)	16/ 46	63.5(7.3)	0.42
Other	7/ 18	61.1(11.5)	12/ 23	47.4(10.5)	0.39
City:					
Ottawa	15/ 53	70.2(6.5)	30/ 66	51.6(6.5)	0.03
Buffalo	19/ 53	63.4(6.7)	18/ 28	32.5(9.6)	0.003
Pittsburgh	3/ 11	68.2(15.4)	11/ 23	51.0(10.6)	
Chicago	6/ 14	57.1(13.2)	6/ 24	74.6(9.0)	
Country:					
Canada	15/ 53	70.2(6 5)	30/ 66	51.6(6 5)	0.03
U.S A	28/ 78	63.0(5 6)	35/ 75	51.9(5.9)	0.12

[a] Excludes Phase II and NCIC Chicago Trial Data
[b] Excludes NCIC Chicago Trial Data

Table 2 summarizes the five year survival rates calculated from the life table analysis. The table shows that the overall five year survival rates for the immunized in comparison with the corresponding controls are highly significant (P=0.01). When the analysis was confined to the randomized patients but restricting to cases where immunization was effective (i.e., excluding the data for the Phase II trial and Chicago data in the NCIC Trial) the results are very highly significant (P=0.002). The survival curve is shown in Figure 1.

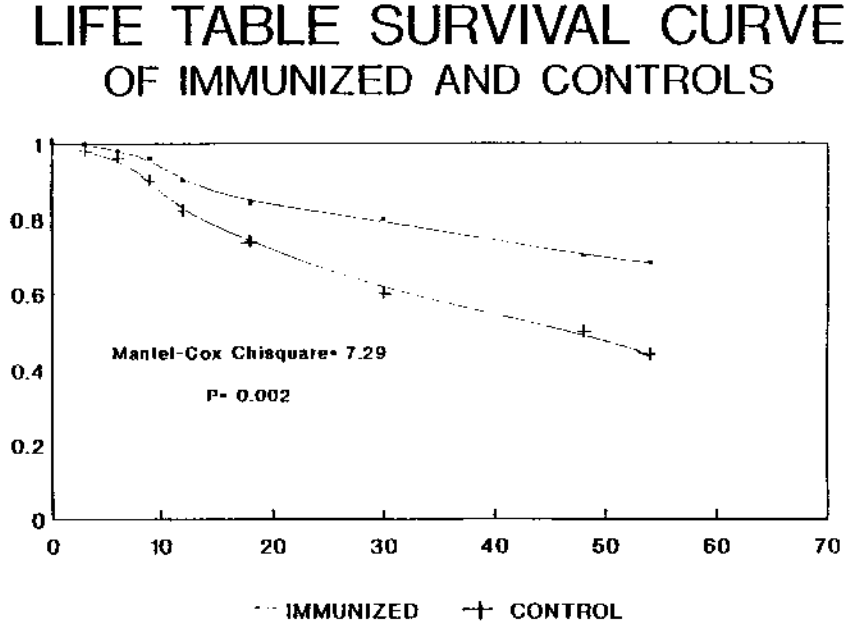

FIGURE 1

Table 2 also shows that if the Phase II trial data were added to the randomized group the survival results are extremely significant (P=0.0007). This group consisting 117 immunized and 117 controls is designated the "Effectively Immunized" as defined previously. The remainder of the table shows the univariate analysis of the five year survival rates of the effectively immunized patients and the corresponding controls categorized by the potentially confounding factors except for histology, city and country which include all the 131 immunized and 141 control patients. The sample sizes were inadequate to detect survival differences for adeno and other histological types. In the analysis by city the results for Pittsburgh was not significant due to samll sample size though it was similar to Ottawa; but Chicago data, though statistically not significant showed a reverse survival trend for immunization and it should be kept in mind that the immunization was not successful in Chicago as measured by the strong DHR criteria. In general the analysis shows that there is about a 30% to 40% increase in five year survival rates among the immunized in comparison with the controls.

TABLE 3
Effect of Immunization on the hazard ratio with adjustment for potentially confounding variables: Cox's Regression Model

Stratification variable	Regression Coefft	(S. E)	Hazard Ratio	95% Confidence Interval
None	-0.7067	0.2105	0.49	0.33 - 0.75
None[a]	-0.6392	0.2417	0.52	0.33 - 0.85
Sex	-0.7042	0.2107	0.49	0.33 - 0.75
Stage	-0.7597	0.2121	0.47	0.31 - 0.71
Histology	-0.7393	0.2143	0.48	0.31 - 0.73
City	-0.8164	0.2204	0.44	0.29 - 0.68

[a] Excludes Phase II Trial data.

To evaluate the effect of immunization after adjusting for the confounding variables a multivariate analysis was performed using Cox's regression analysis. The results are summarized in Table 3. For this analysis all data where immunization was effective (i.e., excluding Chicago data in the NCIC trial) and the corresponding controls were used. The table shows that the estimate of the hazard ratio stratified by each of the confounding variables is about 0.5, indicating that immunization was effective in reducing the risk of mortality by about 50% which is in accord with the power calculations. In all cases the corresponding 95% confidence interval shows that the reduction in mortality due to immunization is statistically significant. The table also shows that the inclusion or exclusion of the Phase II trial data only marginally affect the estimated hazard ratio.

TABLE 4
Distribution of the Induration (mm) to TAA at 12 months among Controls and Immunized at the different centers

Center	Induration (Mean ± S D) Control	Immunized
Ottawa[a]	16.7 ± 15.6 (17)	25.5 ± 18.0 (24)
Ottawa[b]	5.9 ± 7.7 (7)	27.6 ± 16.3 (9)
Ottawa[c]	20.6 ± 16.4 (14)	30.8 ± 20.0 (13)
Pittsburg	17.1 ± 10.1 (8)	21.9 ± 12.5 (9)
Chicago	6.5 ± 9.1 (15)	13.2 ± 22.6 (9)

Numbers in parentheses are the respective sample sizes
[a] Phase II Trial
[b] Phase II Trial patients treated with Methotrexate.
[c] Ottawa NCIC Trial Data.

Table 4 summarizes the distribution of mean induration (mm) to TAA 12 months subsequent to immunization for the immunized and controls at different centres. This information was available for 54 out of the 113 controls and 55 out of the 78 immunized, who survived the first year. Roswell Park Trial did not report DHR at 12 months and is not included in this analysis. Except for Chicago, the immunized patients show strong DHR at 12 months (mean induration ≥20 mm) to TAA. The difference in induration between the immunized and controls is highly statistically significant ($P<0.001$). The controls in Ottawa included in the NCIC Trial show the presence of a strong natural DHR to TAA.

TABLE 5
Distribution of the Induration (mm) to TAA at 12 months among the Controls and Immunized according to survival status

Status	No.	Induration (Mean ± S.D)	95% CL (Scheffè)
Control (dead)	13	17.6 ± 15.2	8.4 to 26.8
Control (alive)	41	13.2 ± 11.9	9.5 to 17.0
Immunized(dead)	13	13.0 ± 8.7	7.7 to 18.3
Immunized(alive)	32	26.2 ± 20.6	18.7 to 33.6

Table 5 shows the analysis of the survival status of the immunized

and controls in relation to the DHR at 12 months. The five year survival data was available for all of the 54 controls and 45 out of the 55 immunized who were skin tested at 12 months. The table also shows the 95% confidence limits (Scheffè) for the different groups. By Analysis of Variance it is found that the immunized alive patients have a very significantly strong DHR ($P<0.001$) in comparison with the controls or the immunized patients who are dead.

III. DISCUSSION

Specific immunotherapy with TAA has been shown to be highly effective in improving the survival probability of stage I and II lung cancer patients. All the three clinical trials considered show a consistent survival advantage in favour of the immunized. The results of the NCIC trial including all patients showed a non significant result for immunization as reported earlier[3]. The same report also showed that the survival curve for the immunized patients in Ottawa and Pittsburgh was distinctly different from the remaining centres. A re-analysis of the NCIC data for Ottawa and Pittsburgh in comparison with their respective non immunized controls showed that the survival curves are marginally significant ($p=0.06$). The lack of statistical significance arises from the small sample size. In this study we have combined the data from three clinical trials to obtain adequate statistical power to enable the detection of the difference in survival. The pooling of the data was in accordance with the criteria listed. Since the patients were randomly allocated in each of the centres following similar protocols the combined analysis is equivalent to that of one large scale multicenter clinical trial. In accordance with the power calculation using the pooled analysis we were successful in detecting a 50% reduction in the mortality risk among the immunized.

For immunization to be effective it is necessary to induce a strong DHR at one year following the immunization. The lack of such immunity as seen from the five year survival rates for Chicago (Table 2) nullifies the survival advantage. This phenomenon was also noticed when the data for the Roswell Park Trial (squamous cell carcinoma) were analyzed as three groups: immunized, adjuvant controls (partially immunized as noted before) and controls. The five year survival rates were 75.0% for the immunized 52.9% for the adjuvant and 32.5% for the control showing a moderate survival advantage for the partially immunized.

The constancy of the relative risk as shown in Table 3 after stratification for the confounding factors shows that the immunization is effective in reducing the risk of mortality in the same proportion irrespective of the influence of other prognostic factors such as sex, stage of cancer, histology or location (city). In other words, though lung cancer survival depends on local treatment conditions, the efficiency of the surgery, stage of the disease and histological type, immunization with TAA appears to confer a doubling of the chance of survival independent of these parameters.

The cause of death was not available for all the deceased patients. However whenever it was available it was due to the recurrence of the cancer. None of the reported deaths were due to auto immune diseases that can be attributed to immunization.

The meta analysis of DHR at 12 months provides evidence that the survival is significantly associated with the induction of strong DHR. In Chicago where the DHR was inadequate the five year survival rates for the immunized were worse than those of the controls (57% vs 75%) though due to the small sample size the results are not statistically significant. The Ottawa immunized group who showed the highest difference in survival rates (70% vs 52%, P=0.03) also were those with the strongest DHR response at 12 months. In particular, 3 out of the 9 immunized treated with methotrexate (Table 4) have tumour growth patterns that strongly suggest a period of dormancy more than ten years after primary surgery which is an extremely rare phenomena (P=0.0006) as we have discussed in detail (This volume, Stewart). An additional 2 out of the 13 immunized (Table 4) showing strong DHR at 12 months included in the NCIC trial have developed dormancy as reported in the same paper and the others are currently being carefully observed, since they have not been followed up for a sufficiently long time for dormancy to be recognized.

In conclusion, the meta analysis has shown the usefulness of immunization with specific TAA in prolonging the survival of lung cancer patients. The increase in survival is independent of other confounding factors as shown by the constancy of the hazard ratio in the multivariate analysis. Finally, the observation of tumour dormancy among the successfully immunized who showed strong DHR at 12 months provides strong biological evidence of the effectiveness of the immunization.

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Cellular Immune Mechanisms and Tumor Dormancy

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