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Successful immunotherapy with *Mycobacterium vaccae* in the treatment of adenocarcinoma of the lung

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ABSTRACT

Immunotherapy with a heat-killed suspension of *Mycobacterium vaccae* (SRL172), given with chemotherapy, in a phase III trial against non-small-cell-lung cancer showed no improvement in the primary endpoint of survival over chemotherapy alone in the initial published analysis. Compliance was poor, with on average only 53% of patients receiving more than 2 injections in the SRL172 arm of the study. Quality of life was, however, improved in those receiving SRL172. Secondary analyses based on compliance with therapy showed that immunotherapy led to significantly improved survival times of patients with adenocarcinoma but, by contrast, had no beneficial effect on survival times of patients with squamous cell carcinoma.

Survival of adenocarcinoma patients receiving SRL172 was increased by a mean of 135 days ($p = 0.0009$, Kaplan–Meier log rank test) and survival after 4 or 5 doses of SRL172 showed a difference of greater than 100 days ($p < 0.05$, Mantel–Hänszel log rank test) in the group receiving SRL172 in addition to chemotherapy.

Despite the problems inherent in a secondary analysis, these results encourage further research on the role of killed preparations of adjuvant-rich micro-organisms, including saprophytic mycobacteria such as *M. vaccae*, and members of related genera in the therapy of a range of cancers.

1. Introduction

There is a very great need in cancer therapy for a treatment that allows good quality of life to be maintained while, at the same time, significantly improving survival time. The intervention also needs, from the perspective of administrators and the state health services, to be simple, safe and cost-effective. The ability of immune reactions induced by infection to cause regression and, occasionally, total remission of established cancers has been known for well over a century.[1] After noticing regressions of sarcomas following attacks of erysipelas Coley, in the USA, developed 'Coley toxins' – extracts of *Streptococcus pyogenes* and *Serratia marcescens* – with which he treated sarcomas, with reported success in some cases.[2] An extensive review published in 1929 suggested that cancer rarely occurred in patients with active tuberculosis,[3] an observation that led to the attempted use of Bacille Calmette Guérin (BCG) vaccine as an immunotherapeutic agent for cancer. More recently, certain infections and vaccinations, including BCG vaccination, have been shown to confer protective immunity against melanoma and a mode of action has been postulated.[4]

A phase III trial of immunotherapy with a heat-killed suspension of *Mycobacterium vaccae* (SRL172), in addition to

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chemotherapy, against non-small-cell-lung cancer showed no significant improvement in the primary endpoint of survival over chemotherapy alone in the initial analysis. The quality of life was significantly improved in those receiving SRL172[5] as assessed by the European Organisation for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire–Core 30.[6] Compliance was poor, with only 53% of the patients receiving more than two injections of SRL172.

In this study, we report re-analyses of this trial with consideration of the histological type of cancer and compliance with immunotherapy.

2. Description of the study

2.1. Patients and methods

The patients whose data we have re-analysed were those recruited into an international phase III trial of immunotherapy with heat-killed *M. vaccae* (SRL172) added to chemotherapy in the treatment of advanced (stage 3B or 4) non-small-cell-lung-cancer.[5] As the great majority of the patients were diagnosed as having squamous carcinoma (50%) or adenocarcinoma (45%), our re-analyses have concentrated on these two types of cancer. Within the trial the patients could receive 3–6 courses of chemotherapy and, if randomised to receive it, 5 injections of SRL172. The SRL172 was to be administered at 28 day intervals, with the first at the time of trial entry. No placebo injections were used.

Non-compliance with the set of injections of SRL172 was partly due to death, partly due to injections not being given when disease progressed and partly because skin reactions to the last injection were still present when the next injection was due to be given.

2.2. Re-analysis 1 (compliers versus non-compliers)

Irrespective of their tumour type, patients were recorded as compliers with immunotherapy if they received all the injections of SRL172 that their survival time allowed, within certain latitudes. These latitudes were that the injections should be received within twice the period at which they should have been administered (i.e. with intervals between doses of no more than 56 days). Patients who completely missed injections despite living long enough to have received them were recorded as non-compliers. Comparisons of survival times were then made for each type of cancer – compliers and non-compliers in the chemotherapy plus SRL172 groups versus survivors in the chemotherapy alone arm. Their significance was assessed by the Kaplan–Meier log rank test and Kaplan–Meier curves were drawn. Since patients randomised to receive chemotherapy only were not given placebo injections, it was not possible to divide them by the same compliance criteria so total survival duration was used.

2.3. Re-analysis 2 (paired analyses between chemotherapy alone and chemotherapy plus SRL172)

For each cancer cell type, five survival-matched subgroups of patients were constructed as set out below:

(A) Patients receiving at least 1 immunotherapeutic injection for comparison with all those receiving chemotherapy alone who entered the study.
(B) Patients receiving 2 injections of immunotherapy, for comparison with those receiving chemotherapy alone who survived for more than 28 days.
(C) Patients receiving 3 injections of immunotherapy, for comparison with those receiving chemotherapy alone who survived for more than 56 days.
(D) Patients receiving 4 injections of immunotherapy, for comparison with those receiving chemotherapy alone who survived for more than 84 days.
(E) Patients receiving 5 injections of immunotherapy, for comparison with those receiving chemotherapy alone who survived for more than 112 days.

The 'residual' survival, or survival beyond the point for which each subgroup was defined, was determined. That is, for example, survival beyond day 56 for the third group and day 84 for the fourth group. Furthermore, these residual survival patterns were compared for 365 days after the minimal survival point. For these analyses Mantel–Hänszel log rank statistics were used. Adjustments were made by means of Cox's proportional hazard model, for covariates of possible prognostic significance; namely, gender, age, use of tobacco and disease stage at entry.

3. Results

3.1. Demographic details of patients

Age, sex, stage of disease and smoking status data on the compliers and non-compliers are shown in Table 1. There were no significant differences between the two groups of adenocarcinoma patients and the only difference between the groups of squamous carcinoma patients was that the non-compliers were 4.1 years younger than the compliers ($p < 0.05$).

3.2. Re-analysis 1 (compliers versus non-compliers)

Overall the survival time of patients with either tumour type who did not comply with immunotherapy did not differ from their respective chemotherapy control groups; $p < 0.16$ by Kaplan–Meier log rank test.

Patients in the squamous carcinoma complier group lived no longer than did their chemotherapy control arm – 303 compared with 307 days; $p = 0.9911$ by Kaplan–Meier log rank test (Fig. 1a).

Patients with adenocarcinoma who complied with immunotherapy lived on average 135 days (around 4.5 months) longer than the control group receiving chemotherapy alone – 357 (95% confidence intervals (CIs) 293–421) days compared with 222 (95% CI 180–263) days; $p = 0.0009$ by Kaplan–Meier log rank test, a 60% survival advantage (Table 2, Fig. 1b).

3.3. Re-analysis 2 (paired analyses between chemotherapy alone and chemotherapy plus SRL172)

Results of analysis of the survival-matched subgroups of patients with adenocarcinoma are shown in Table 3. The *p*-val-

Table 1 – Demographic data on the patients studied

	Compliers ($n = 45$)		Non-compliers ($n = 21$)
Adenocarcinoma			
Age	60.2 ± 8.3	ns	63.4 ± 8.8
Sex: M/F	27/18	ns	11/10
Stage: IIIA/IIIB/IV	2/13/30	ns	3/4/14
Smoking: yes[a]/no	27/18	ns	8/13
	Compliers ($n = 61$)		Non-compliers ($n = 25$)
Squamous carcinoma			
Age	62.9 ± 8.0	$p < 0.05$	58.8 ± 9.3
Sex: M/F	49/12	ns	18/7
Stage: IIIA/IIIB/IV	6/22/33	ns	2/10/13
Smoking: yes[a]/no	36/25	ns	19/6

a Present and past smokers.

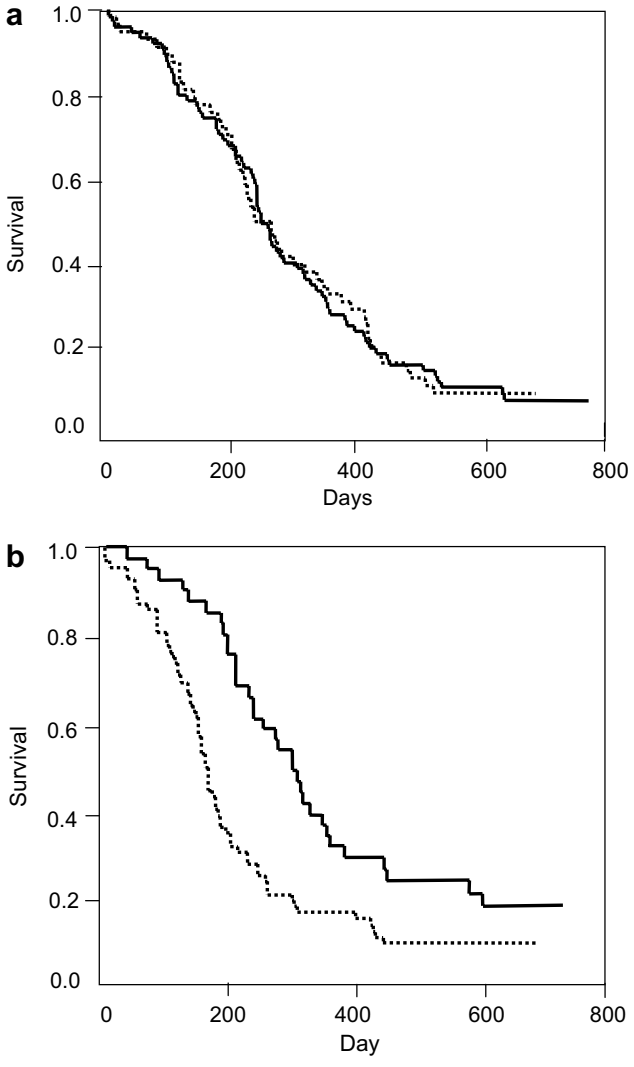

Fig. 1 – Kaplan–Meier graphs illustrating the differences found between the responses of those who complied with immunotherapy and those receiving chemotherapy alone. (a) Squamous carcinoma patients and (b) adenocarcinoma patients. ——, compliers with immunotherapy; ·····, chemotherapy control group.

ues from the unadjusted Mantel–Hänszel tests and those from the proportional hazards regression model are similar and suggest an increasing survival advantage for patients as they received more injections of *M. vaccae*, becoming significant for those receiving 4 or 5 injections. The proportional hazards regression model also suggests a survival advantage for females, for patients with an earlier stage of disease at trial entry and for non-smokers.

None of these analyses showed any difference between the groups when applied to patients with squamous carcinomas (Mantel–Hänszel log rank test $p > 0.5$) for which the data are not shown.

4. Discussion

The re-analyses of the phase III study presented here were not specified in the original study protocol but were undertaken as a result of a trend towards improved survival in the patients with adenocarcinoma revealed in the planned analysis. There were also concerns that a failure of compliance with the immunotherapy by some patients may have obscured its impact on survival times.

Although the *p*-values of the unadjusted and adjusted re-analyses were similar, intrinsic differences and confounding factors other than those included in the regression model were possible. Accordingly, this re-analysis must be regarded as hypothesis-generating rather than hypothesis-confirming, since exploratory analysis of patient subgroups can produce misleading results.[7] Notwithstanding, both types of analysis showed survival benefits of 100 days or more for adenocarcinoma patients receiving 4 or more injections of *M. vaccae*, whereas no improved survival was found by either type of analysis amongst patients with squamous carcinoma.

It is unlikely that the very considerable differences in the observed therapeutic efficacy of *M. vaccae* immunotherapy between patients with the two subtypes of carcinoma are attributable to confounding factors or trial design problems. Table 1 shows that there were no significant differences in the demographic factors measured between adenocarcinoma and squamous carcinoma patients or between compliers and non-compliers within either cancer sub-type that could

Table 2 – The results obtained from the first sub-analysis of data on adenocarcinoma patients

Complied with immunotherapy ($n = 45$)			Chemotherapy controls ($n = 72$)		
Survival time (days)		Standard error	Survival time (days)		Standard error
Mean	357	33	Mean	222	21
Median	302	24	Median	177	7
Percentiles			Percentiles		
25%	450	119	25%	249	40
50%	302	24	50%	166	7
75%	210	12	75%	112	15

Comparative survival analysis by Kaplan–Meier log rank test: statistic 10.96; degree of freedom 1; significance $p < 0.0009$.
Although the data are not shown, there was no difference between the chemotherapy alone patients and those who complied poorly with the immunotherapy schedule (Kaplan–Meier log rank test: statistic 1.33; degree of freedom 1; significance $p = 0.2487$).

Table 3 – The results obtained from the second sub-analysis of data on adenocarcinoma patients

Subgroup	Chemotherapy + *M. vaccae*			Chemotherapy only			Significance of difference (p)		Significant covariates (p)
	No.	Days of survival		No.	Days of survival		Mantel–Hänszel log rank	Adjusted for Cox's model	
		Total	Residual[a]		Total	Residual			
A	67	211	211	75	168	168	0.2009	0.2980	Gender 0.0122 Grade[b] 0.0325
B	60	268	240	72	198	170	0.1411	0.1947	Gender 0.0062
C	48	332	276	69	236	180	0.0816	0.0872	Gender 0.0387
D	37	384	300	66	267	183	0.0121	0.0119	
E	27	416	304	59	308	196	0.0414	0.0525	

a Survival beyond the time-point of the comparison (this was the point at which the next injection was due).
b Amount of disease at the time of entering the study.

explain the differences. Thus, although preliminary in nature, the results obtained by this re-analysis raise important questions; namely, the mode of action of an immunotherapeutic agent containing both antigens and adjuvants and the marked difference in response by two different subtypes of lung cancer. These questions are considered in the light of recent developments in immunology in a separate paper.[8]

Research on dosing schedules of SRL172 is also required. A likely schedule, already in use in our studies, is frequent administration of *M. vaccae* early in the treatment to overrule the Th2-enhancing influence of cancer cell products. Once tumour growth rate is slowed or stopped, the frequency of *M. vaccae* doses can be reduced. An additional valuable property of such immunotherapy is its virtual lack of adverse side effects and improved quality of life.

Conflict of interest statement

None declared by the authors, though C.A.S. and J.L.S. are shareholders of SR Pharma Plc, and J.L.S. is a Scientific Director of Stanford Rook Ltd.

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