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An evaluation of a preparation of *Mycobacterium vaccae* (SRL172) as an immunotherapeutic agent in renal cancer

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ARTICLE INFO

Article history:
Received 23 July 2007
Accepted 2 November 2007

Keywords:
Renal cell carcinoma
Immunotherapy
Mycobacterium vaccae
SRL172

ABSTRACT

Two studies were carried out to evaluate heat-killed *Mycobacterium vaccae* SRL172 as an immunotherapeutic agent for patients with metastatic, post-nephrectomy, renal cell carcinoma. In the first study, 60 patients in France and the UK received injections of SRL172, and their survival was compared with that of historical controls who had been treated either with biological response modifiers (IL-2, IFN-α) or chemotherapy. In the second study, 36 patients were randomised to receive treatment with IL-2 alone or IL-2 plus SRL172. Survival and adverse events related to the treatments were assessed and compared between treatment groups.

The first study showed that those treated with SRL172 alone survived equally as long as those receiving IL-2 or IFN-α and both treatment groups survived longer than those on chemotherapy ($p < 0.001$), a result supported by Cox's proportional hazards regression analysis. The second study, stopped early due to drug supply issues, showed that the addition of SRL172 to IL-2 made no difference to survival compared to IL-2 alone, in the limited numbers treated. Adverse events occurring in those receiving SRL172 in the first study were mild and in the second study those receiving IL-2 alone had significantly more adverse events than those receiving SRL172 plus IL-2 ($p < 0.001$).

It is concluded that SRL172 may have activity in metastatic renal cancer and has very low toxicity, making it worthy of further study.

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2007.11.003

1. Introduction

There are over 4000 new cases a year of renal cancer in Europe and though early disease is curable, patients with metastatic disease have a median survival of less than 12 months.[1–3] Until recently, biological response modifiers (BRMs) such as interferon-alpha (IFN-α) and interleukin-2 (IL-2) were the standard treatment for good performance patients with metastatic renal cell carcinoma. IFN-α has a response rate of 10–20%,[4–6] with an average duration of response of approximately 6 months[4] and gives a modest improvement in overall survival.[4–6] IL-2 has shown overall response rates of 15–35% in metastatic renal cancer with phase II data showing that high dose bolus IL-2 can give durable remission in a small subset of patients.[7–10]

The development of multi-targeted tyrosine kinase inhibitors has changed the therapeutic landscape and these drugs are rapidly becoming the standard of care for these patients.[11,12] Currently, the new role of BRMs has yet to be defined; however, there is evidence that response to the 'targeted' TKIs is associated with modifications in immune responses[13] and therefore exploration of BRMs, particularly less toxic agents, remains valuable.

Since 1882 when Coley used a preparation of *Streptococcus pyogenes* and *Serratia marcescens* ('Coley toxins') to treat sarcoma[14] there has been evidence that bacterial products can beneficially affect the course of several cancers. Bacille Calmette-Guérin (BCG) has been used extensively with some definite but limited anti-tumour efficacy. In an attempt to improve the immune response in patients with tuberculosis, several mycobacteria have been screened for their ability to stimulate protective immune responses. One of these, a strain of *Mycobacterium vaccae* prepared as a heat-killed suspension (SRL172), expresses heat shock proteins and cross-reactive bacterial products involved in the tumour antigen presentation as well as being targets for anti-tumour cytotoxic T cell responses[15,16]. In pre-clinical models with mice,[17,18] and in patients,[19,20] immunotherapy with SRL172 has also been shown to stimulate a cytokine profile reflecting a Th1 immune response and suppression of Th2 responses, a pattern postulated to promote cell-mediated anti-tumour immunity.[21]

Small phase II studies of SRL172 in melanoma and prostate cancer show some evidence of a Th2 to Th1 switch similar to those reported for tuberculosis patients receiving SRL172.[19,20,22–24]

Phase II studies in advanced thoracic cancers showed a trend towards improved overall median survival in patients treated with chemotherapy and SRL172 compared to chemotherapy alone.[25–27] Subsequent phase III studies, however, failed to show a benefit in overall survival with SRL172 in combination with chemotherapy compared to chemotherapy alone although there was a significant improvement in the quality of life of patients who received SRL172.[28,29] Post hoc sub-group analysis showed that immunotherapy significantly prolonged average survival in those with adenocarcinoma.[30]

With the evidence of responsiveness of RCC to biological response modifiers, we have conducted two studies of SRL172 in renal cancer, a non-randomized phase II study (Study A) to test the safety and efficacy of SRL172 in patients with advanced renal cell carcinoma and a randomized phase II trial (Study B) to compare IL-2 alone and with SRL172 in patients with metastatic renal cell carcinoma and here report on the results of these two serial studies.

2. Description of the studies

2.1. Study A

2.1.1. Study design

This multi-centre prospective single arm phase II study was carried out at five centres in the UK and France in patients with locally advanced or metastatic renal cell carcinoma. The primary objective was to determine the response rate (RR) of SRL172 and secondary objectives were to determine the toxicity, progression free survival (PFS) and the overall survival (OS).

2.1.2. Patient selection

Eligibility criteria included histologically confirmed, advanced or metastatic RCC with measurable disease, previous nephrectomy and WHO performance status 0–2. Patients were aged 18 years or more and had adequate, renal, hepatic and cardiac function and haematological indices.

Patients were ineligible if they had brain metastases, previous other malignancies within the last 5 years, or it had received previous systemic therapy for metastatic RCC. Patients who were receiving, or had received systemic steroids within the last 6 weeks were excluded. Previous hormone therapy was allowed, provided there was evidence of progression on treatment or it had been withdrawn for more than 6 months.

2.1.3. Selection of historical controls

Historical controls, extensively described in previous studies,[31,32] consisted of patients with RCC treated with chemotherapy between 1975 and 1984, IL-2 (five studies between 1986 and 1990) and IFN-α (three studies). A total of 1185 patients were available before exclusion in this historical control population. From this group, patients were selected if they met the inclusion criteria in Section 2.1.2.

2.1.4. Treatment and assessment

SRL172 is a suspension of heat-killed *Mycobacterium vaccae* NCTC 11659 in borate buffered physiological saline. Each 0.1 ml of SRL172 contained 1 mg wet weight, equivalent to 10^9 bacilli. Patients received 0.1 ml of SRL172 intradermally on entry and after 2, 4, 8, 12, 16, 20 and 24 weeks. Patients then entered into the maintenance phase receiving injections every 8 weeks. Treatment was discontinued if patients had progressive disease, unacceptable adverse effects or they withdrew consent.

All patients had pre-treatment clinical assessment, baseline biochemistry, haematology and CT scanning. At each visit, patients were assessed for toxicity using NCIC toxicity criteria. Objective tumour responses were assessed by WHO response criteria based on eight weekly CT scans.

2.1.5. Statistical analysis

PFS and OS were measured from first treatment and live patients censored at the last date seen. Both efficacy and safety analyses were conducted on the set of intention-to-treat (ITT) patients, defined as all patients who received at least one dose of the study medication.

Kaplan–Meier survival analyses were conducted on the ITT population and historical control groups. The historical control patients were separated into two groups, one containing those who received IFN-α or IL-2 and the other containing patients in chemotherapy trials. The log-rank test was used to test if patients in the study and historical control groups had differing survival.

A Cox proportional hazards regression analysis was used to make comparisons between the study and historical control groups, adjusting for any differences in the distribution of prognostic factors. The age, performance status and delay between diagnosis and treatment were included in the model, together with terms for the interactions of these factors with the study group to test whether there were differential effects of these prognostic factors in the two groups.

2.2. Study B

2.2.1. Study design

This second study was a prospective multi-centre, randomized Phase II study in patients with advanced renal cancer. Patients were randomized to receive either single agent subcutaneous IL-2 or a combination of IL-2 and SRL172. The primary end-point was TTP, and secondary end-points were toxicity, RR and OS.

2.2.2. Patient selection

Inclusion and exclusion criteria were as above except that non-nephrectomised or previously treated patients were allowed. Previous resistance to IL-2 therapy was a contraindication.

2.2.3. Treatment and assessment

Subcutaneous IL-2 was administered at 9 million IU daily (4.5 million IU twice daily) for 5 d a week for 6 consecutive weeks. In non-progressing patients, this 6 week cycle was repeated after a 14-d rest. Following this, patients received maintenance therapy consisting of 5 d treatment every month. Patients randomized to SRL172, received an intradermal injections of 1 mg two weeks prior to commencement of IL-2 therapy, 2 weeks later, then at four weekly intervals. Treatment was continued until disease progression, unacceptable toxicity, a decline in EORTC performance status >3 or withdrawal of consent.

Safety and efficacy assessment was as in study A.

2.2.4. Statistical analysis

TTP and OS and RR were defined as above. Efficacy analysis was conducted on all randomized patients and safety analysis on all patients who received at least one dose of the treatment. Recruitment of 66 patients was planned to give a power of 0.8 to detect an improvement of median progression free survival (with one-sided $a = 0.1$) from 4 months on IL-2 treatment alone to 7 months on IL-2 combined with SRL172. The planned size of the study was such that only large differences would be detectable. It was intended that any positive findings from this study would be confirmed in a larger follow-up study.

3. Results

3.1. Study A

3.1.1. Patient characteristics

The patient characteristics of the 60 patients recruited into the study are shown in Table 1.

3.1.2. Treatment

All of the 60 patients received at least one injection of SRL172 and are included in the efficacy analysis. Fifty-six out of the 60 patients received three doses of SRL172 but, as one had a major protocol violation, 55 patients are included in the treatment analysis. Twenty patients completed the 24 week treatment phase and 15 of these continued on maintenance injections. Eight of these patients completed the 24 week maintenance phase and continued with the post-maintenance phase treatment.

3.1.3. Safety

Three hundred and eighty nine injections were given to 60 patients. Most of the adverse effects were mild or moderate and are depicted in Table 2 with injection site reactions being most common, occurring in 16 patients (26.7%) with mild abscesses developing in 2 and a moderate abscess developing in the third. Mild or moderate flu-like symptoms were seen in 9 (15%) patients and mild asthenia in 15 (25%). One patient (1.7%) experienced severe asthenia and fevers which improved spontaneously. There were no changes in haematological or biochemical parameters due to the injections.

Table 1 – Study A patient characteristics

Characteristic	
Sex	
Male	42 (70%)
Female	18 (30%)
Median age (range)	58 years (38–80)
Median time from diagnosis	19.6 months (0.8–280.6)
Median time from nephrectomy	19.6 months (0.7–280.6)
Histology	
Clear cell carcinoma	52 (87%)
Other	8 (13%)
Performance status	
0	33 (55%)
1	23 (38%)
2	4(6%)
Sites of disease	
Lung	43 (71%)
Liver	11 (18%)
Bone	10 (17%)
Lymph node	23 (38%)
Adrenal	7 (12%)
Soft tissue	4 (7%)
Other	17 (28%)

Table 2 – Study A: adverse effects related to SRL172

Adverse effect	% and number of patients effected			
	Grades 1–2		Grades 3–4	
Asthenia	25%	15/60	1.7%	1/60
Fever/chills	5%	3/60	1.7%	1/60
Sweats	5%	3/60	–	
Flu-like syndrome	15%	9/60	–	
Myalgia	1.7%	1/60	–	
Pain at injection site	5%	3/60	–	
Injection site reaction	26.7%	16/60	–	
Injection site abscess	5%	3/60		
Nausea	10%	6/60	–	

3.1.4. *Efficacy*

No complete remissions were seen and 1 partial remission occurred. Seven (11.7%) patients had stable disease during the treatment phase. Fifty-one patients had progressive disease during the treatment or maintenance phase of the study.

The median overall time to progression was 2 months. The median overall survival was 13 months (range 0.5 months – not reached). Of note, the 2-year survival was 29% (Fig. 1).

3.1.5. *Comparison of SRL172 with historical controls*

Of the 1185 historical control patients screened, 610 had received chemotherapy, 327 had received IL-2 and 248 had received INF-α. After exclusion of 425 patients for non-nephrectomy, previous systemic therapy, PS > 2 or CNS metastases, age <18 years) 760 (318 chemotherapy, 442 biological therapy) were included in the comparison with SRL172. Patient characteristics are shown in Table 3.

The age distribution and sex ratios in the SRL172 group were similar to those in the historical control groups. Performance status was similar between the biological therapy groups and SRL172 group, but the chemotherapy trials included a higher proportion of patients with a performance status of 2 (17%, compared to 6% of the SRL172 group). Time from diagnosis to treatment was similar between the historical control groups, but longer for the SRL172 group (medians of 8 and 9 versus 19 months, respectively).

The overall survival of the SRL patients was compared to historical controls (Fig. 2). There were no statistically significant differences between the SRL172 group and the biological therapy group (Log-rank test $p = 0.54$) but the survival of the patients from the chemotherapy studies was significantly worse than those receiving SRL172 or biological therapy (Log-rank test $p < 0.001$).

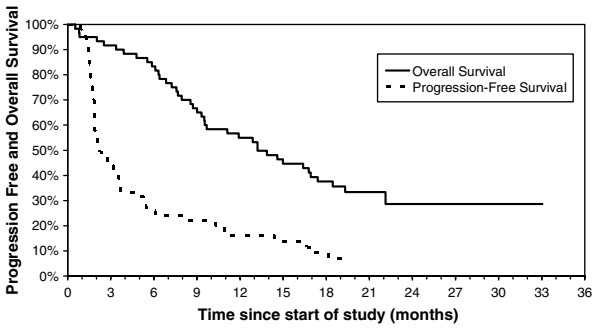

Fig. 1 – Progression free and overall survival (Study A).

To try and adjust for the differing proportion of patients with different risk factors in the different patient groups, we adjusted for performance status and time from diagnosis to treatment; Cox's Proportional Hazards Regression analysis was applied, to compare the study groups after adjustment for prognostic factors including performance status and time from diagnosis to treatment, in relation to survival.[33] The time from diagnosis to treatment was considered either by dividing into less than, and greater than, 24 months or by taking the time from diagnosis as a numeric variable. In both cases, the estimates of the study differences were little altered from the analysis without adjustment. The results of the model are illustrated in Fig. 2b, which shows predicted survival curves, after centring on the averages of the prognostic factors over all studies. The three curves are marginally closer together than the unadjusted curves, which reflects the adjustment made for the worse distribution of prognostic factors in the historical controls, particularly in the chemotherapy group (Fig. 3).

3.2. *Study B*

This study was closed before the planned recruitment was reached because SRL172 production and supply was not sustained.

3.2.1. *Patient characteristics*

Patient characteristics are shown in Table 4. Data on clinical response, progression free survival and overall survival was available on 36 patients.

3.2.2. *Treatment*

Eighteen patients were randomized to each arm of the study. One patient in each arm did not receive the drug after randomization and these were included in the efficacy analysis but excluded from the safety analysis. Two patients in the IL-2 arm and 3 patients in the IL-2 + SRL arm withdrew due to toxicity without completing one cycle. Seven patients in the IL-2 arm and 6 patients in the IL-2 + SRL arm received more than one cycle of treatment.

3.2.3. *Safety*

Seventeen patients from each arm were included in the safety analysis. Overall, there was less toxicity in the IL-2 + SRL arm

Table 3 – Study A: characteristics of SRL treated patients and historical controls

	Biological therapy		Chemotherapy		SRL172	
Total	444	100%	318	100%	60	100%
Age (years)						
⩽60 yrs	293	66%	210	63%	36	60%
>60 yrs	149	34%	117	37%	24	40%
Median age	55		57		58	
Range	21–76		24–82		38–80	
Sex						
Female	139	31%	93	30%	18	30%
Male	303	69%	225	70%	42	70%
Performance status						
0	226	51%	102	32%	33	55%
1	195	44%	160	50%	23	38%
2	21	5%	56	18%	4	7%
Time from diagnosis to treatment						
<24 months	319	72%	230	72%	35	58%
⩾24 months	123	23%	88	28%	25	42%
Median	8		9		19	
Range	0–237		0–169		0–279	
Lung metastases						
Absent	133	30%	52	16%	17	28%
Present	309	70%	266	84%	43	72%
Liver metastases						
Absent	353	80%	261	82%	49	82%
Present	89	20%	57	18%	11	20%
Bone metastases						
Absent	346	78%	223	70%	50	83%
Present	96	22%	95	30%	10	18%
Other metastases						
Absent	209	47%	235	74%	19	32%
Present	233	53%	83	26%	41	68%

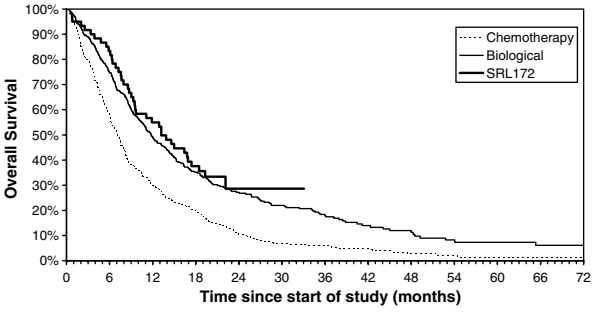

Fig. 2a – Overall survival SRL172 treated versus historical controls (Study A).

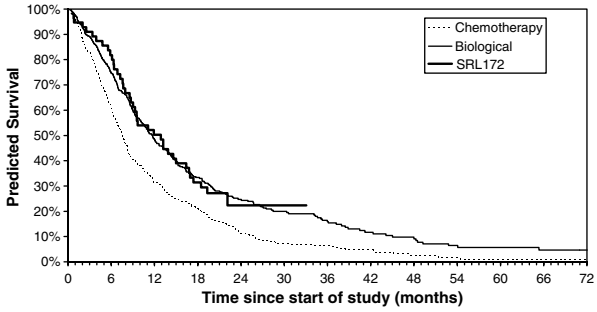

Fig. 2b – Predicted survival (Study A). (Correcting for PS and time from diagnosis to start of study.)

compared to the IL-2 alone arm, apart from a single case of angio-oedema. There were no changes in haematological or biochemical parameters related to the injections. As shown in Table 5 there was less asthenia ($p < 0.002$), flu-like syndrome ($p < 0.02$) and vomiting ($p < 0.009$) amongst those receiving SRL172, and trends of reduction in nausea and dyspepsia ($p < 0.09$) and in the IL-2 injection site inflammation ($p < 0.07$). The overall difference in toxicity between the two treatment groups was statistically significant in favour of the combination ($p < 0.001$; Fisher's exact test).

3.2.4. *Efficacy*

Three patients in the IL-2 arm and 4 patients in the IL-2 + SRL arm withdrew before completing one cycle of treatment and were not assessed for radiological response. Five patients (28%) in the IL-2 arm and 7 patients (39%) in the IL-2 + SRL arm achieved stable disease. The rates of progressive disease in the IL-2 and IL-2 + SRL arms were 44% and 50%, respectively. None of these differences achieved statistical significance. Complete and partial responses were not observed in this cohort of patients.

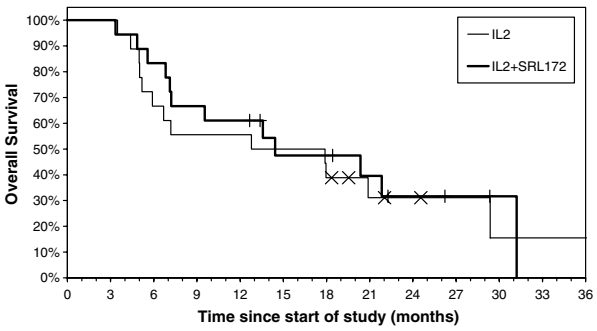

Fig. 3 – Overall survival for interleukin 2 ± SRL172 (Study B).

In the IL-2 alone group the median time to progression was 72 d (CI 62–182) and in the combination group it was 77 d (CI 63–89), with no significant difference between the groups (Log-rank test $p = 0.47$). In the IL-2 alone group the median overall survival was 390 d (CI 171 – not estimable) and in the combination group it was 301 d (CI 208–450), with no evidence of a significant difference between the groups (Log-rank test $p = 0.91$).

4. Discussion

In this paper, we have assembled all of the clinical research data for SRL172 in renal cancer to help the decision about pursuing this potentially useful approach alone or in combination. We recognise that the data are incomplete and all conclusions must be cautious.

The large phase II study (Study A) with comparison to the historical database suggests that immunotherapy with SRL172 may be comparable against renal cell carcinoma as are the established biological response modifiers, IFN-α and IL-2, and effective than chemotherapy. Adding SRL172 to a course of subcutaneous IL-2 appeared to make no difference to efficacy in a small randomised phase II (study B), but may reduce toxic side-effects.

In study A we used a historical control population which included only those patients with prior nephrectomy, no prior systemic therapy and PS. < 2. Overall the populations were reasonably matched but the proportions of patients with separate risk factors were different. We have attempted to adjust for this as much as possible and the survival differences are still apparent.

Table 4 – Study B: patient characteristics

	IL-2		IL-2 + SRL172		Both groups	
Total patients	18	100%	18	100%	36	100%
Age (years)						
⩽60 yrs	12	67%	10	56%	22	61%
>60 yrs	6	33%	8	34%	14	39%
Median age	55		60		58	
Range	48–69		45–58		45–69	
Sex						
Female	6	33%	1	6%	7	19%
Male	12	67%	17	94%	29	815
Performance status						
0	7	39%	10	56%	17	47%
1	11	61%	8	44%	19	53%
Time from diagnosis to treatment						
<24 months	2	11%	3	17%	5	14%
⩾24 months	16	89%	14	78%	30	83%
Missing data			1	5%	1	3%
Histology						
Clear cell	14	78%	18	100%	32	89%
Other	4	22%	0		4	11%
Prior systemic treatment						
IFN	14	78%	13	72%	27	75%
IFN + IL-2 + 5FU	1	6%	1	5%	2	6%
Other IFN combinations	2	11%	3	17%	5	14%
Other	1	6%	1	6%	2	6%
Sites of metastases						
Lung	13	72%	13	72%	26	72%
Liver	3	17%	3	17%	6	17%
Bone	2	11%	5	28%	7	19%
MSKCC risk factors for second line therapy						
0	8	44%	2	11%	10	28%
1–2	9	50%	15	83%	24	67%
⩾3	1	6%	0		1	3%
Missing data			1	6%	1	3%

Table 5 – Study B: adverse effects related to the combination of IL-2 and SRL172 and IL-2 alone

Adverse effect	IL-2 + SRL172		IL-2 alone	
	Grades 1–2	Grades 3–4	Grades 1–2	Grades 3–4
Asthenia	47% 8/17	6% 1/17	83% 14/17	18% 3/17
Fever/chills/sweats/flu-like syndrome	53% 9/17	6% 1/17	94% 16/17	–
Nausea/dyspepsia	35% 6/17	–	59% 10/17	6% 1/17
Vomiting	6% 1/17	–	47% 8/17	–
Diarrhoea	29% 5/17	–	53% 9/17	–
Abdominal cramps	–	–	29% 5/17	6% 1/17
Anorexia	12% 2/17	6% 1/17	24% 4/17	–
Peripheral oedema	6% 1/17	6% 1/17	–	6% 1/17
Angio-oedema	–	6% 1/17	–	–
Injection site inflammation	18% 3/17	–	41% 7/17	6% 1/17

Studies based on historical controls have various well known disadvantages and it is difficult to make comparisons of quality of life, but toxic side-effects of BRMs are well known. In contrast, patients on SRL172 experienced only mild local reactions and minimal disruption of their lives (see Table 2). Thus, while the survival times of those receiving SRL172 and BRMs were similar, definite advantages of the former are its ease of administration, its low cost and its lack of adverse effects on quality of life.

Study B was stopped early due to supply of SRL172. We therefore have attempted to ascertain the certainty with which we have excluded a strong therapeutic effect of the adding SRL172. Cox's Proportional Hazards Regression analysis was applied to the PFS data to determine the likelihood of detecting a large difference such as a 50% increase in TTP. Such an increase in median progression free survival is approximately equivalent to a hazard ratio of 0.67 assuming roughly exponential distributions and proportional hazards. The estimated hazard ratio for IL-2 and SRL172 against IL-2 alone was 1.29 (0.63–2.65 at 90% confidence and 0.7 and 2.63 at 95% confidence), thus the true hazard ratio is unlikely to be as low as 0.67. Similarly, for overall survival, median survival was 72 d in the IL-2 alone arm and a 50% increase would therefore be 108 d. As the 95% confidence interval for the IL2 + SRL172 arm was between 63 and 89 d, this increase is excluded, implying that the true median in the combination arm is unlikely to be as large as this. Thus, even had the trial been as large as was planned the likelihood of finding a 50% difference would have been remote.

Although no advantage in efficacy of IL-2 + SRL172 over IL-2 alone was seen, there were less overall adverse effects in the IL-2 + SRL172 group ($p < 0.001$) (Table 5). Injection with SRL172 or other mycobacteria induces significant changes to T cell subsets and so is possible that the toxicity seen when IL-2 is injected alone is due to the lack of simultaneous release of a range of other substances, which modulate this toxicity. The complete panoply of cytokines and other substances induced by *M. vaccae* may help to control the toxic aspects of application of the pure cytokine and thus the observed reduction in toxicity. This observation suggests a possible role, requiring further clinical investigation, for *M. vaccae* as an adjunct in therapy with biological response modifiers.

In conclusion, these studies show that SRL172 is well tolerated and suggest that it may offer the survival benefit at a similar level to established cytokine therapy in patients with renal cancer, with the additional benefit of low toxicity. In addition, there are data to suggest that SRL172 reduces the adverse effects of biological therapy with subcutaneous IL-2. Taken with other evidence that SRL172 modifies the course of several cancers, including adenocarcinoma of the lung,[30] and recent advances in immunology that have facilitated the development of a hypothesis to explain its anti-tumour activity,[34] we suggest that SRL172 warrants further examination in renal cancer therapy.

Conflict of interest statement

None declared.

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