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Killed *Mycobacterium vaccae* suspension in children with moderate-to-severe atopic dermatitis: a randomized, double-blind, placebo-controlled trial

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Summary

Background The hygiene hypothesis is often proposed to explain the high prevalence of atopy in the western world. Dysregulation of the immune system may result from inadequate exposure to micro-organisms such as mycobacteria. A small trial suggested that a killed extract of *Mycobacterium vaccae* ameliorates atopic dermatitis (AD).

Objectives To confirm in a large clinical trial whether killed *M. vaccae* ameliorates AD in 5–16-year-old children.

Methods This was a randomized, placebo-controlled, double-blind, multi-centre study of the effect of intradermal injection of killed *M. vaccae* (0.1 or 1 mg) on patients, aged 5–16, with moderate-to-severe AD. Patients were followed up for 24 weeks. The primary end point was the change in severity of AD at 12 weeks, assessed using the six area, six-sign, atopic dermatitis (SASSAD) score. Secondary end points included changes in disease extent, patient's global assessment and children's dermatology life quality index.

Results There were 166 patients randomized. The mean SASSAD score fell to a similar degree at week 12 in all treatment arms: from 33 to 24, (26%) in the high-dose group, from 30 to 23 (25%) in the low-dose group and from 36 to 27 (24%) in the placebo group ($P > 0.05$).

Secondary end points followed the same trend. Adverse events were generally those expected to occur in this population. Injection site reactions occurred in 32 patients at week 4.

Conclusions *M. vaccae* was no more effective than the placebo in ameliorating the severity of AD.

Keywords atopic dermatitis, double blind, hygiene hypothesis, *Mycobacterium vaccae*, placebo-controlled trial, randomized, SASSAD score

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Introduction

Although the aetiology of atopic dermatitis (AD) remains uncertain, it is now thought to involve dysfunction of regulatory dendritic cells and T cells that normally prevent excessive pro-inflammatory responses (hypersensitivity reactions) [1–3]. In acute eczematous skin lesions the pro-inflammatory response is largely of a T helper (Th)2 type, whereas in more chronic lesions both Th1

and Th2 responses are involved [4]. Maturation of regulatory dendritic cells and T cells depends on exposure to environmental microbes [5]. Lack of priming of these cells by microbes during childhood resulting in exaggerated immune responses is considered to be the immune basis of the hygiene hypothesis of atopic diseases [5–7].

Based on the above hypothesis, reduced exposure to saprophytic environmental mycobacteria with increasing

urbanization might be expected to contribute to the recent surge in atopic diseases. Furthermore, therapeutic exposure to appropriate mycobacterial antigens may have the potential to improve atopic illness. In murine models, such exposure does indeed seem to demonstrate the potential to down-regulate Th2 responses [8] but currently it remains to be established whether similar mechanisms might operate in human atopic disease.

There are currently conflicting data as to the importance of these mycobacteria in suppressing allergic diseases [9]. A study of Japanese schoolchildren in 1997 showed a strong inverse association between delayed hypersensitivity to *Mycobacterium tuberculosis* and asthma [10] and prompted increased recent interest in this potential area. An independent study of 418 South African children showed a similarly strong negative association between tuberculin reactivity and allergic rhinitis [11], although another large study of 606 children in Crete found no significant association between mycobacterial skin reactivity and atopic diseases [12]. Some of these epidemiological studies may suggest a link between mycobacterial reactivity and atopic disease, but they do not address the question as to whether administration of mycobacterial antigens can modify the clinical course of these conditions.

In order to more directly address this question a number of placebo-controlled clinical trials have been performed over the last 5 years. Many of these studies have been small and results have been mixed. In a small single-centre randomized, double-blind, placebo-controlled trial (RDBPCT) of 41 children aged 5–18 years, administration of the environmental mycobacterium *Mycobacterium vaccae* (*M. vaccae*) significantly ameliorated the severity of AD (surface area affected) compared with the control group [13]. However a follow-up study examining the effect of *M. vaccae* in younger children aged 2–6 years old showed that although there was a 38–54% reduction in surface area affected after *M. vaccae* administration, there was a similar improvement in the placebo group [14]. This study illustrated the natural tendency for children's AD to improve with time and thus the important need for control data in assessing the potential therapeutic benefit of any medicine particularly in this condition. Three small clinical trials in adults with moderately severe asthma showed that administration of this environmental mycobacterium is not associated with a statistically significant reduction in symptoms [15–17].

To provide more definitive information as to the possible therapeutic value of *M. vaccae* in the treatment of AD, we set up a multi-centre RDBPCT of school age children with moderate-to-severe disease. The primary aim was to test the hypothesis that intradermal administration of killed *M. vaccae* significantly ameliorates the severity of AD in these children.

Methods

Trial design

This was a double-blind, placebo-controlled trial, conducted at 19 centres in the United Kingdom and Croatia, to compare two doses of *M. vaccae* (SRP299) in treatment of childhood AD. The study was co-ordinated and monitored by the sponsor, SR Pharma. The study comprised three sequential phases; a screening visit, followed after not more than 4 weeks by a randomization and treatment visit, and then a 24-week 'follow up' phase. Patients returned to the clinic for four follow-up visits at weeks 4, 8, 12 and 24.

M. vaccae SRP299 is a slightly cloudy suspension whereas the placebo was a clear, colourless liquid. Furthermore, in previous studies most patients developed a delayed hypersensitivity reaction (slight swelling and erythema) at the injection site, with some patients developing a small ulcer that heals to leave a residual scar. Therefore, a number of steps were taken to reduce observer bias. An independent evaluator, who was blinded to the treatment that the patient received, determined the extent and severity of AD. The patient/parent was requested to cover up the injection site with a sticking plaster or other dressing before attending the centre at weeks 4, 8, 12 and 24. This was done to conceal any local reaction resulting from the injection, from study personnel other than the injection site inspector. The study medication was administered by a designated person who was not involved in the blinded assessments, and who was not allowed to discuss injection site reactions with the independent evaluator. An injection site inspector examined the patient for local reactions (the medication administrator and the independent site inspector could be the same person). The results of the local reaction site inspection were recorded on a separate log. Any local reactions resulting in an adverse event were also recorded separately. By following these procedures, the objective and subjective study assessments were performed by people who were not aware of the treatment that the study patients received. Treatment allocation remained concealed until after the database was locked.

Subjects

Subjects were males and females aged between 5 and 16 years, with a diagnosis of AD as defined by the diagnostic criteria of the United Kingdom Atopic Dermatitis Working Party.[18] Disease severity was required to be moderate to severe as defined by the Rajka and Langeland criteria.[19] During the course of the study it became clear that these severity criteria did not correlate well with the six area, six sign, atopic dermatitis (SASSAD). There was concern that inclusion of subjects with a low SASSAD score might reduce the power of the study. The protocol was therefore

amended to include only subjects with a SASSAD score of at least 20. Females of child-bearing potential were required to provide a pre-treatment negative serum or urine pregnancy test at baseline and to agree to practise effective birth control during the study.

Patients with clinically infected eczema at baseline, those with a history of serious adverse drug reaction to any drug and those who had received any vaccine, drug, or device for investigational use within 3 months before randomization were excluded.

Treatment

The study medication was given as a single 0.1 mL intradermal injection over the upper third of the deltoid muscle at the randomization visit. A central randomization code was computer generated for this study by the sponsor, assigning patients at random to one of three treatment groups to be treated with 1 or 0.1 mg of killed *M. vaccae* suspension SRP299 or placebo (phosphate buffered saline). Patients were randomized in blocks of six. At the randomization visit each patient was assigned a sequential three-digit patient number by the investigator from a list of randomization numbers that had been supplied to the investigator. The patient number corresponded to the number on the patient pack of study medication.

Patients were required not to have made any change to the treatment for their eczema for 4 weeks before the study and were required not to use topical or systemic immunomodulatory agents (including tacrolimus, pimecrolimus, ciclosporin, methotrexate, systemic steroids), traditional Chinese medicines or phototherapy during the study or the previous 3 months. Patients were otherwise allowed to use their standard AD medication throughout the study, including topical steroids. Changes in topical treatment were not permitted, although subjects were allowed to adjust the frequency and quantity of topical steroid applied as required.

Assessments

The primary efficacy end point in this study was the change in severity of AD as assessed by the SASSAD severity score,[20] between baseline (week 0) and week 12. In order to ensure that investigators were adopting a uniform approach to the use of this parameter a training session was undertaken at a meeting of investigators before the study commenced. SASSAD scoring was demonstrated and practised using projected pictures of affected body areas. Secondary efficacy parameters included the change in SASSAD at 24 weeks and changes in affected body surface area assessed using the rule of nines. Patient's provided a global assessment of response on a five-point scale ranging from much better to much worse. Pruritus severity was monitored using the follow-

ing five-point scale at each visit: all the time, very much, a lot, a little, not at all. Sleep disturbance was monitored using the following four-point scale: very much, a lot, a little, not at all. Frequency of use of topical corticosteroids was monitored using the following five-point scale: twice a day, once a day, alternate days, occasionally, none. Changes in quality of life were assessed using the children's dermatology life quality index (CDLQI).[21]

In addition, injection site reactions and adverse events were recorded.

Statistics

Statistical analysis was performed by SR Pharma. The primary efficacy parameter was analysed using analysis of variance and analysis of covariance models, and differences between treatment arms were estimated using least squares means. To ensure the robustness of the results the primary efficacy analysis was repeated using the non-parametric Wilcoxon rank sum test. The associated 95% confidence intervals for the difference between treatment arm median changes were derived using the Hodges-Lehmann method.[22]

Published data on mean change from baseline in SASSAD scores suggest standard deviations of between 14 and 24 U for this end point, with baseline scores ranging from 40 to 70 U on the SASSAD scale.[20] The minimum clinically significant change in this study was considered to be a 20% reduction in SASSAD score from baseline. Based on these estimates, a sample size of 40 patients per treatment arm was estimated to ensure that the study had at least 80% power to detect a statistically significant treatment effect.

Ethical considerations

The protocol was approved by the multi-centre research ethics committee for Scotland and the appropriate local ethics committee or Institutional Review Board (IRB) for each participating centre. Written informed consent was obtained from parents of every participating patient and additionally from the patients themselves if they were sufficiently mature.

The trial was performed in accordance with the principles expressed in the Declaration of Helsinki.

Results

Demographics

Demographic data are summarized in Table 1. A total of 166 patients were randomized. Of these, 155 completed the study. The patient flow is summarized in Fig. 1.

Table 1. Demographic data

	High-dose group <i>N</i> = 56	Low-dose group <i>N</i> = 58	Placebo group <i>N</i> = 52
Age (mean, SD, range)	9.6, 3.6, 5–16	9.3, 3.2, 5–16	9.6, 3.6, 4–16
Gender (male/female)	26/30	31/27	29/23
Duration of AD in years (mean, SD, range)	8.9 3.8, 1.4–16.5	8.8 3.3, 1.4–15.2	8.8 3.7, 2.4–16.9
Baseline SASSAD (mean, SD)	33, 15	30, 13	36, 13
Percentage of skin involved (mean, SD)	40, 18	36, 18	45, 20
Numbers of patients using topical steroids during the trial (as and when required), classified* as none, mild, moderate, potent and very potent	4, 4, 16, 30, 2	4, 8, 7, 38, 1	5, 2, 14, 31, 0

*As classified by the British National Formulary.

AD, atopic dermatitis; SASSAD, six area, six-sign, atopic dermatitis.

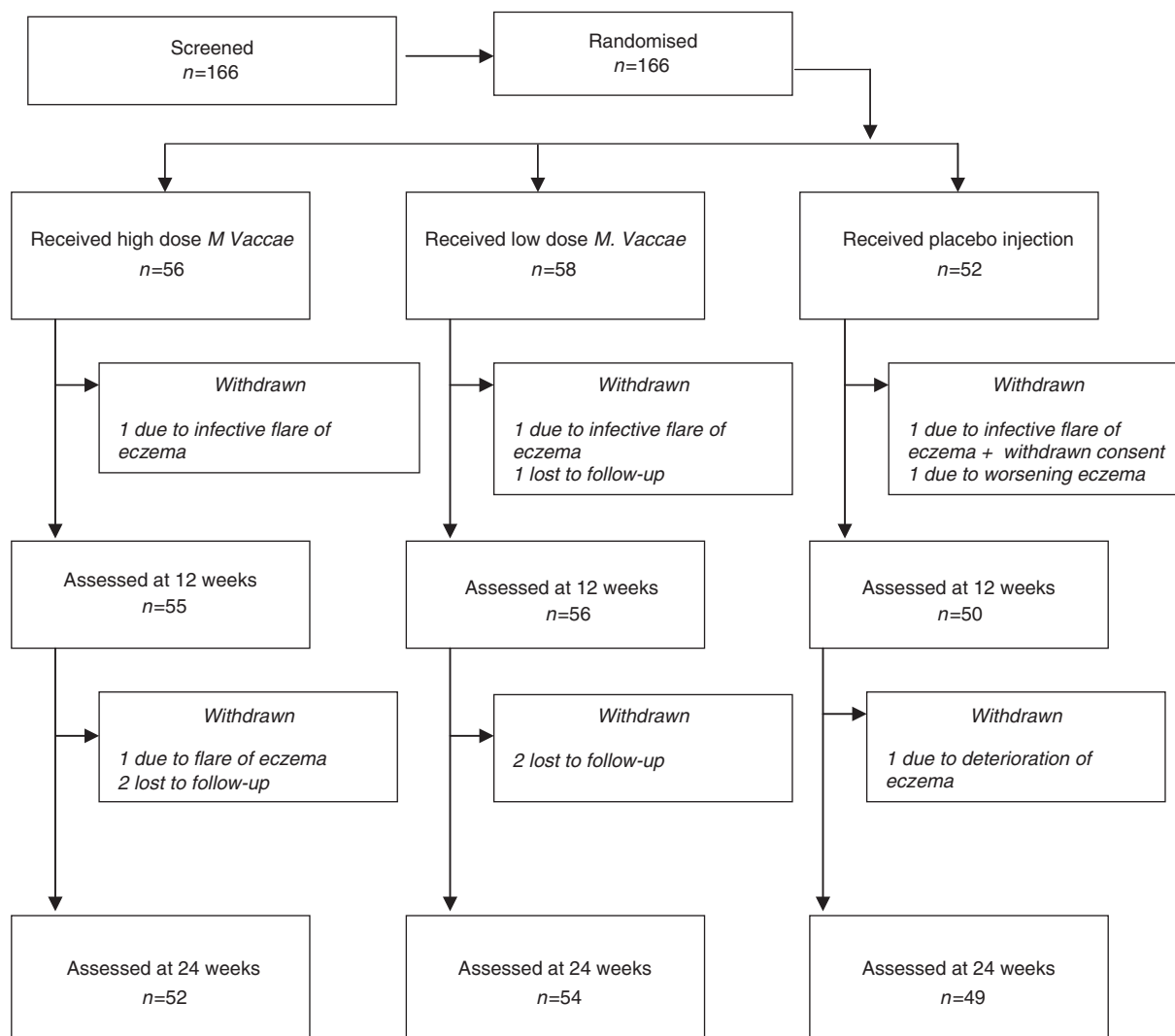


Fig. 1. Patient flow chart.

Changes in eczema severity

Changes in the mean SASSAD scores are shown in Fig. 2. There was a decrease in SASSAD score from baseline over

time, and the general pattern was similar for all three treatment arms. At 12 weeks (the primary end point) the mean SASSAD score had improved by 9.4 points (26%) in the high-dose group, 7.0 points (25%) in the low-dose

group and 8.8 points (24%) in the placebo group. Therefore the difference in the improvement in the mean scores between the high-dose group and placebo was 0.6 points in favour of the high-dose treatment (95% confidence interval (CI) -4.3 to 5.4 , $P > 0.05$). In the low-dose treatment group the difference from placebo was 1.8 points in favour of placebo (95% CI -3.0 to 6.6 , $P > 0.05$). There were no statistically significant differences between the changes observed in the three treatment groups at any time-point.

The mean change in percentage body surface area affected between week 0 and week 24 was -13.0% in the high-dose group, -12.0% in the low-dose group and -15.1% in the placebo group. None of the comparisons between treatment groups was statistically significant at any time-point.

There was no statistically significant difference between the groups at any time-point with respect to the

patient's global evaluation of response. At week 24, the number of patients who felt 'much better' or 'slightly better' was 34 (65% of 52 patients) in the high-dose group, 36 (67% of 54 patients) in the low-dose group and 32 (65% of 49 patients) in the placebo group.

There were no significant differences between the groups with respect to itching at any time-point.

Sleep disturbance demonstrated no significant difference between groups except at week 8. At this time-point there were statistically significant comparisons in favour of *M. vaccae* between the low-dose and placebo groups ($P < 0.05$) and the two active groups combined vs. placebo ($P < 0.05$).

Changes in topical steroid use showed no significant differences between treatment arms (Fig. 3).

CDLQI scores were improved in all three treatment groups. At week 24 the improvements reached 4.8 points in the high-dose group ($n = 50$), 5.4 in the low-dose group ($n = 52$) and 5.5 in the placebo group ($n = 47$). The differences between groups were not statistically significant.

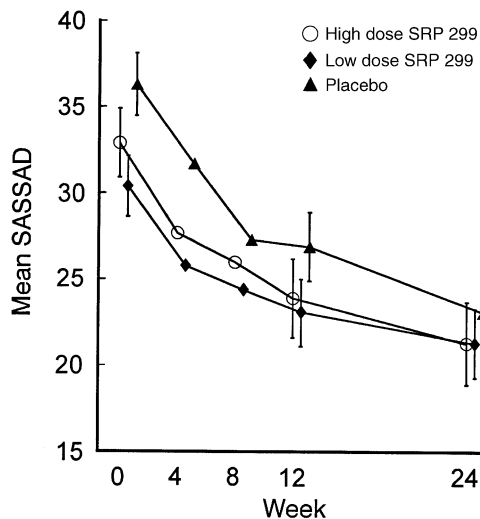


Fig. 2. Changes in mean SASSAD score in each group.

Adverse events and safety monitoring

Of the 166 patients in the safety population, 103 (62%) reported a total of 260 adverse events. The most commonly reported adverse events were eczema (53 patients), followed by infected eczema (24 patients), asthma (17 patients) and upper respiratory tract infection (14 patients). The incidence of adverse events was similar in the three treatment groups.

Forty-one (25%) patients reported one or more adverse events that were classified by the investigator as at least possibly related to study medication. The most common of these was eczema (13% patients), with similar numbers in each treatment group.

Five patients experienced serious adverse events (so defined because the events resulted in hospitalization and

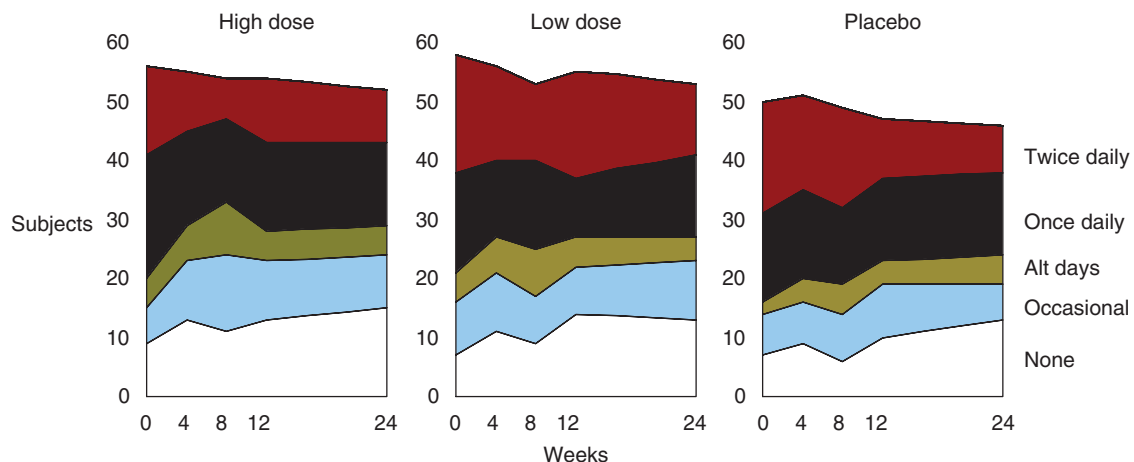


Fig. 3. Usage of topical corticosteroids assessed at each visit using a five-point scale.

probably not related to the trial medication): three patients in the high-dose group (two exacerbations of eczema and one upper abdominal pain) and two patients in the placebo group (one infected eczema and one hospitalization after a fall).

The most common injection site reactions were induration and erythema, with 32 patients experiencing these reactions at week 4. In general, the incidence of injection site reactions was dose related. The number of patients with an injection site reaction decreased with each visit, and the reactions resolved over the 24-week study period. Vesiculation and ulceration were uncommon and, where they occurred, they resolved rapidly. There was no swelling of the injected arm and only one episode of infection. One patient, who received high-dose *M. vaccae*, developed a haematoma at the injection site that resolved without treatment.

Discussion

This study was a parallel-group assessment of two doses of *M. vaccae* vs. placebo in patients with moderate-to-severe AD. The three groups were reasonably well balanced at baseline with respect to demographic data and severity of AD. Overall, the efficacy analyses do not suggest a difference between the effects of *M. vaccae* and placebo on SASSAD scores for AD. The observed reductions from baseline in mean SASSAD scores occurred in all three treatment arms, but the differences between the treatment arms were not statistically significant. In the calculation of the sample size it was determined that the study would have 80% power to detect a 20% reduction in this score. There was at least a 20% reduction in SASSAD scores from baseline in all three treatment groups. The protocol amendment that sought to increase power by recruiting patients with a minimum SASSAD score of 20 might have resulted in an increased tendency to recruit subjects who were flaring rather than at average levels of activity, so spontaneous improvement followed.

The 95% CIs for the differences in changes in SASSAD between the groups would indicate that a large treatment effect is unlikely; however, the data do not exclude the possibility that *M. vaccae* exerts a rather modest therapeutic effect on the severity of AD. Given the low toxicity of this treatment, a modest level of response might still be of clinical value. By 24 weeks the improvements in mean SASSAD score had reached 35% in the high-dose group, 30% in the low-dose group and 36% in the placebo group. These data do not indicate a trend for either of the treatment groups to improve further over time relative to placebo.

The patterns of safety data were broadly similar on the active treatment arms and in the placebo arm. There was no suggestion of any treatment-related mild exacerbation of the disease, fever or malaise, which are common adverse effects of vaccines. This might support the lack

of evidence for an immune response but does not explain the significant placebo response.

The 1 mg dose of SRP299 administered in this study was the same as the dose of killed *M. vaccae* suspension SRL172 used in a previous study on AD study in 5–18 year olds, and used extensively in tuberculosis, cancer and allergy studies. The intradermal route of administration was chosen as this delivers *M. vaccae* to the antigen-presenting cells in the skin. This route has been extensively used in previous studies in tuberculosis, cancer and allergy. Both the doses used and route of administration have been shown to be well tolerated for both single and multiple doses.

The findings in this study are virtually identical to those of Arkwright and David [14]. In this study, which investigated children aged 2–6, there was again a marked improvement in patients receiving placebo as well as those treated with *M. vaccae*.

The results of the current study do not confirm the improvement relative to placebo observed in an earlier and smaller study by Arkwright and David in children aged 5–18 years.[13]

In conclusion, the efficacy analyses did not suggest a difference between the effect of *M. vaccae* SRP299 and placebo on SASSAD scores for AD. The safety data were reassuring, with no indication in of any effect attributable to *M. vaccae*, except for reactions at the study drug injection site.

It remains possible that there may be a therapeutic role for certain purified components of mycobacteria. Perhaps extraction of active constituents which produce the stimulation of RegT function previously demonstrated by *M. vaccae* [23,24] may allow a more consistent clinical response to be achieved. Finally, there are emerging data indicating that administration of *M. vaccae* by the oral route may be more influential in modulating immune function [25] and future studies will be needed to explore this in atopic eczema.

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Conflicts of interest: GAWR is a shareholder and Research Director of SR Pharma, which holds intellectual property related to the clinical use of *M. vaccae* and its derivatives. PDA has undertaken previous research sponsored by SR Pharma.

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