**Vaccine Research International Plc**
**Reg. no. 4449300**

# Phase I Clinical Trial of Vaccine SA75 against Staphylococcal Infections

## Synopsis of Trial

### Design of Trial

A Phase I Clinical Trial in healthy male volunteer subjects assessed the safety, tolerability and antibody response to VRi Vaccine SA75. Forty eight healthy male subjects were randomly assigned to one of three groups; each group received a different dose level of vaccine and within each group of sixteen subjects, twelve received vaccine and four received placebo. The trial was double-blinded where neither subjects nor medical or administrative staff knew which subjects received vaccine or placebo; four vaccinations were given at two weekly intervals.

The first group received the lowest dose of vaccine and progression to the higher dose levels for the second and third groups was based on a review of safety data obtained for the previous group. At regular intervals after each vaccination, each subject attended the outpatient clinic where general medical status and reactions to the vaccination were recorded with blood sampling for haematology, biochemistry and Staphylococcal-specific antibody testing.

### Results

***Local Tolerability***

Following each vaccination, the vaccination site was examined at 0hr, 4hr and 8hr on day 1 which was the day of the vaccination, followed by days 2, 3 and 8 after each vaccination and record made of itching, bruising and pain on a scale of absent, mild, moderate and severe and in case of induration and erythema on a scale of 1-4 and a numerical value was ascribed according to the level of the adverse event.

Two subjects were withdrawn from the trial on account of local adverse effects following the first vaccination but pleasingly made a full recovery by seven days following this vaccination.

As anticipated, local reaction to vaccination in terms of discomfort, pain, itching, erythema and induration occurred in most vaccinated subjects; bruising was recorded but was an infrequent occurrence with no difference between vaccinated and placebo subjects.

***Immune Response***

Immune response was evaluated using immunoblotting and enzyme-linked immunosorbent assay (ELISA) methods. Immunoblotting measures antibody reactivity to individual polypeptides in the bacteria by visualisation as a series of horizontal bands; ELISA is a less specific method which measures total antibody reactivity as a reactive serum dilution (titre).

The sera were tested against homologous organism and binding proteins namely collagen binding protein (GST-Can), clumping factor (His-Clf), fibronectin binding protein (GST-D) and extracellular adherence protein (Eap) known to be present on the organism.

- Immunoblotting

Immune response was assessed by the number of new or increased intensity bands where over 4 was designated 'strong', 0-4 was 'weak' and no change was 'absent'. For IgG (immunoglobulin class G) reactivity, 24 of 32 subjects (75%) demonstrated a strong response after the fourth injection compared to none of the placebo subjects ($p<0.0001$). Inclusion of weak responders with the strong responders resulted in 31 of 32 (97%) of vaccinated subjects compared to 5 of 12 (42%) placebo subjects being classified as responding ($p<0.0001$). Further research is required to evaluate the significance of 'weak responders'.

There was a detectable effect of dosage with 6 of 11 strong responders after the fourth vaccination in the low dose group (0.15 mg), 8 of 10 strong responders in the intermediate dose group (0.36 mg) and 10 of 11 strong responders at the highest dose (0.45 mg). While there was a significant immune response following one vaccination at even the lowest dose the number of strong responders increased from the first to the last vaccination from 13 of 36 (36%) strong responders at first vaccination to 24 of 32 (75%)

following the fourth vaccination (p 0.001). There was thus a general increase in response in relation to the number of vaccinations with a suggestion of a plateau effect between the third and fourth vaccinations at the medium and high doses.

- ELISA

ELISA responses were assessed by the number of subjects whose ELISA titres doubled or more compared to their own pre-vaccination titre. There was a significantly greater number of positive values considering all dose levels where 23 of 36 (64%) vaccinated subjects had a positive ELISA response compared to 1 of 12 (8%) of placebo subjects (p 0.0009).

There was no detectable difference in ELISA titres in relation to different dose levels of vaccine but there was a significant increase in positive responses in the highest dose level (0.45 mg) following only the first vaccination with 20 of 36 subjects (55.5%) compared to none of the placebo subjects (p 0.0007).

- Antibodies to Binding Proteins

Collagen binding proteins and other proteins may be important in stimulating antibodies which will protect against Staphylococcal infection; these antibody levels may serve as a 'proxy' marker of protective efficacy of the vaccine. By the end of the vaccination course, 21 out of 33 vaccinated subjects (64%) demonstrated positive titres (i.e. a greater than two fold increase above pre-vaccination levels) against collagen binding protein compared to 1 of 12 (8%) placebo subjects (p 0.005).

There was a trend of increasing numbers of subjects who had collagen binding protein antibodies with successive vaccinations ranging from 13 of 35 (37%) following the first vaccination to 21 of 33 (64%) following four vaccinations (p 0.03). There was only one subject in the placebo group who demonstrated a positive titre against collagen binding protein (8%).

Only 3 vaccinated subjects demonstrated antibodies to clumping factor (His-Clf) and against extracellular adherence protein (Eap) while none demonstrated antibodies against fibronectin binding protein (GST-D). One subject in the placebo group demonstrated antibodies against all four binding proteins.

**Conclusion**

No significant adverse reactions were observed at the lower and intermediate vaccine dose levels which were thus considered acceptable for vaccination of human subjects. There was significant immune response to both homologous organism and putatively protective proteins at the low dose level with increased levels of response at the intermediate and high dose levels. Immune response was obtained following first vaccination at each dose level with a generally increasing response following the third or fourth vaccinations; it may therefore be possible to reduce dosage and number of vaccinations to obtain a satisfactory level of protection against this infection.

VRi Plc will now proceed to further clinical studies for example further refinement of vaccination schedules in both male and female subjects – to allow definitive efficacy trial of the vaccine in patients at risk of this infection followed by acquisition of product licence.

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