

## ANTI-IL-4 CONJUGATE VACCINE PREPARED WITH PFENEX CRM197 IS MORE IMMUNOGENIC THAN KLH ANALOG

Anti-interleukin 4 (IL-4) conjugate vaccines consist of IL-4 chemically coupled to a carrier protein. Immunizations with these vaccines, in combination with an adjuvant, elicit the production of polyclonal anti-IL-4 neutralizing antibodies in mice. We aimed to evaluate two widely used carrier proteins, namely Pfenex's CRM197 (a non-toxic diphtheria toxin mutant) and KLH (keyhole limpet hemocyanin), by manufacturing one vaccine with each carrier protein and comparing their immunogenicity in mice.

BALB/c mice received four intramuscular injections of murine IL-4 conjugate vaccine (prepared with CRM197 or KLH) or PBS, all emulsified with a squalene oil-in-water adjuvant. Blood collections were performed before dosing and at days 39, 60 and 120. Serum samples of immunized mice were assessed for the presence of anti-muLL-4 antibodies by ELISA and their muLL-4 neutralizing capacities (NC50) evaluated in a proliferative cellular bioassay using CTLL-2 cells.

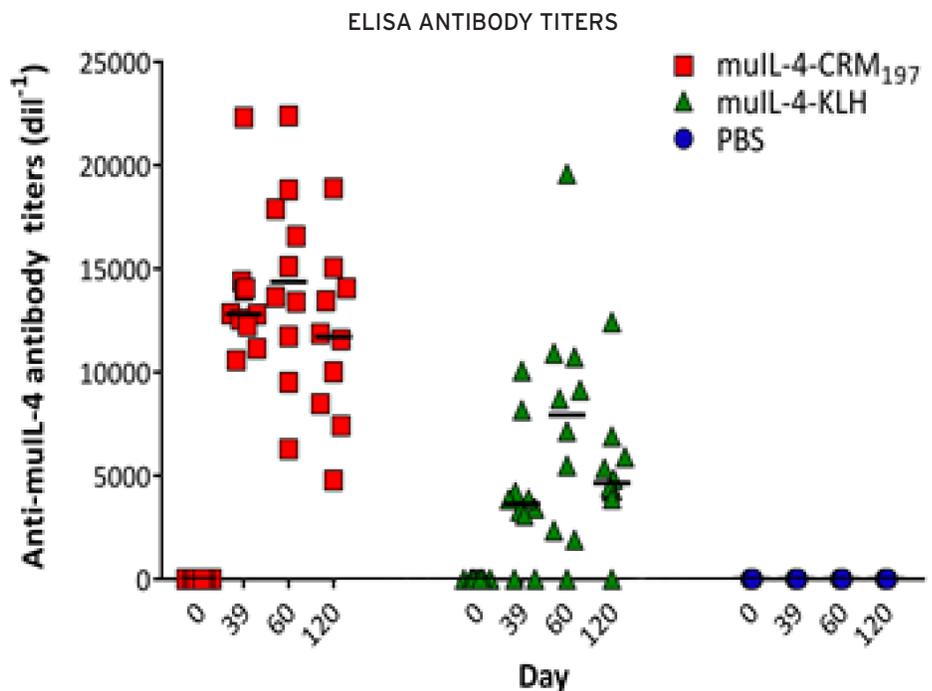
In the group receiving the vaccine prepared with CRM197 (muLL-4-CRM197), anti-muLL-4 antibodies were detected in all mice (10/10), at all timepoints postimmunization, with a peak of the response at day 60 (median titer: 14364 dil-1).

In mice immunized with the vaccine prepared with KLH (muLL-4-KLH), the overall levels of anti-muLL-4 antibodies produced were weaker, with a peak of the response at day 60 (median titer: 7933 dil-1).

In this group, some mice (3/10) failed to produce anti-muLL-4 titers at all timepoints post-treatment. As expected, no muLL-4 binding antibodies were detected before dosing, or in the PBS control group at all timepoints. The neutralizing

capacities of the anti-muLL-4 antibodies were stronger in the group of mice immunized with the vaccine prepared with Pfenex's CRM197 versus KLH, with a greater number of responder mice at all timepoints postimmunization.

These results indicate that Pfenex's CRM197 is superior to KLH as a carrier protein in muLL-4 conjugate vaccines, in both the quantity of anti-muLL-4 antibodies produced (antibody titers) and their quality (neutralizing capacities).



### BIOASSAY NEUTRALIZING CAPACITIES

	MUIL-4-CRM197				MUIL-4-KLH				PBS			
	D0	D39	D60	D120	D0	D39	D60	D120	D0	D39	D60	D120
<b>RESPONDERS (NC50 &gt; 200 DIL-1)</b>	0/10	5/10	7/10	9/10	0/10	2/10	4/10	0/5	0/5	0/5	0/5	0/5