V17. YELLOW FEVER VACCINE, RECOMBINANT ENVELOPE PROTEIN (rYFE), PLANT DERIVED, FORACTIVE IMMUNIZATION: PRECLINICAL STUDIES IN MICE AND MONKEY MODELS.

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INTRODUCTION Although there is a widely used and highly effective attenuated live vaccine to combat yellow fever, there are very rare cases of serious adverse events associated with the vaccine. Therefore, in collaboration with Fraunhofer USA (FhCMB), Bio-Manguinhos / Fiocruz, is developing a plant-derived subunit vaccine for prevention of yellow fever. The vaccine candidate, termed YFE-1T, is based on the envelope protein (YFE) from Yellow fever virus strain 17DD. The vaccine candidate is produced in Nicotiana benthamiana plants, purified, characterized in vitro and in vivo. FhCMB utilizes transient expression technology based on an Agrobacterium tumefaciens type vector to rapidly produce high levels of YFE target protein in hydroponically grown fresh leaf biomass. We believe that the use of plants could provide a safer supply of proteins than natural or animal-based sources and may contribute a cost advantage over traditional bioreactor technology.

OBJECTIVE Evaluation of virus neutralizing antibody response and cellular responses in animal models with each of two adjuvants: aluminium hydroxide adjuvant (Alhydrogel™) and an experimental saponin-based adjuvant.

METHODOLOGY For challenge study C57BL6 mice were used at 4 weeks of age. Animals were vaccinated with YFE-1T dose-escalation of 5μg; 1μg; 0,2μg and 0,1μg with both Alhydrogel and saponin-based adjuvants. The vaccination schedule was on days 0 and 28 with blood collection for plaque reduction neutralization test (PRNT) always two days before the application of the antigens. The intracerebral challenge with 17DD strain virus occurred on day 42. For monkey study, 22 rhesus macaques (*Macaca mulatta*) were vaccinated with 10μg or 30μg with both Alhydrogel and saponin-based adjuvants. The vaccination schedule was on days 0 and 30 with

subcutaneous challenge on day 90. Samples were collected on days -3; 29; 60; 88 and 105 and viremia evaluated by qPCR and plaque assay.

RESULTS After the second vaccine dose, the groups inoculated with saponin-based adjuvant had a significant increase ($p < 0.05$) in mean titers of neutralizing antibodies to YF. However, only the group, that received the highest dose of antigen, showed a mean titer above the cutoff point for seropositivity (2.6 log10), significantly higher than the control group vaccinated with the commercial attenuated vaccine, which obtained a mean titer of 1.86 log10. The survival rate, in the mice challenge study was 100% for the groups with 5μg YFE-1T dose for both adjuvants. Nevertheless, the group that received 5μg dose with saponin-based adjuvant did not develop symptoms related to infection of 17DD substrains virus in a murine model, while the group that received the same dose with Alhydrogel developed symptoms, such as claudication and paralysis of hind limbs, and ocular discharge.

CONCLUSION The results suggest that YFE-1T protein is a possible candidate vaccine against yellow fever to be evaluated in a Phase I Clinical Trial.

KEYWORDS Yellow Fever, plant-derived subunit vaccine, pre-clinical studies.