

## Dr Françoise Guinet-Morlot

### G protein content measured by ELISA correlates with immune response: results from a randomized dose- ranging Phase II study

**F. Guinet-Morlot, P. Riou, S. Pichon**

Sanofi Pasteur has a longstanding commitment to constantly improve quality standards for its commercialized vaccines. The next generation of rabies vaccine PVRV-NG2 has been developed with an improved and innovative manufacturing technology resulting in a highly purified vaccine that lacks antibiotic and components of human and animal origin. As part of global efforts to reduce animal experimentation and to comply with Sanofi Pasteur internal 3R policy, the ambition is to register PVRV-NG2 without any tests on animals. As of today, all in vivo tests have been replaced by in vitro alternatives, except for the NIH test (based on mice immunization followed by intracerebral viral challenge). This NIH test currently measures human rabies vaccine potency, a key criterion for vaccine release, since it is considered to be predictive of an adequate immune response in humans (1). To replace this NIH test, we develop an ELISA (2) able to quantify Glycoprotein G and detect structural alterations (3). This ELISA is used to monitor the process, follow the vaccine stability, and define at the formulation step the final antigen content per dose of vaccine.

To select the dose of antigen in the PVRV-NG2 formulation we compared in a randomized controlled Phase II dose ranging study (NCT03145766) the immune response of 3 batches of PVRV-NG2 at a low, medium, and high antigen content with the licensed Imovax® rabies vaccine and with a previous formulation named, PVRV-NG1.

In this descriptive study, 320 healthy adults were vaccinated according to a 5-dose, post-exposure regimen at day (D)0, 3, 7, 14, and 28 by intramuscular (IM) route. All adults received human rabies immunoglobulin (HRIG) at D0 (IM route, simulated conditions). Participants were followed for 6 months after final vaccination and immunogenicity was assessed using the RFFIT test. Results showed that at each time-point, the geometric mean titers (GMTs) increased with PVRV-NG2 antigen content, and the highest dose of PVRV-NG2 investigated compared favorably to Imovax® Rabies.

This study demonstrates a dose effect of antigen content at all time-points, and confirms that G protein content measured by ELISA was an accurate predictor of the human immune response. This test was selected by an International Working group under the leadership of EPAA to be the most promising candidate to replace the NIH test (4) and EDQM is currently organizing an international collaborative study for assessing the transferability and the intra- and inter-laboratory variability of this test. Upon successful completion of this study, this ELISA will be introduced as an alternative to the NIH method in the Pharmacopoeia monograph.

(1):WHO TRS 941

(2) Chabaud-Riou M, Moreno N, Guinchard F, Nicolai MC, Niogret-Siohan E, Sève N, Manin C, Guinet-Morlot F, Riou P. G-protein based ELISA as a potency test for rabies vaccines. *Biologicals*. 2017 Mar;46:124-129

(3) Audrey Toinon, Nadege Moreno, Heloise Chausse, Emilie Mas, Marie Claire Nicolai, Fabien Guinchard, Isabelle Jaudinaud, Françoise Guinet-Morlot, Patrice Riou and Catherine Manin, Potency tests to discriminate between differentially over-inactivated rabies vaccines: Agreement between the NIH assay and the Sanofi Pasteur ELISA, submitted for publication

(4) Sylvie Morgeaux, Bertrand Poirier, C. Ian Ragan c., Dianna Wilkinson d, Ulrich Arabin e, Françoise Guinet-Morlot f, Robin Levis g, Heidi Meyer h, Patrice Riou f, Shahjahan Shaid e, Dmitriy Volokhov g, Noël Tordo i, Jean-Michel Chapsal, Replacement of in vivo human rabies vaccine potency testing by in vitro glycoprotein quantification using ELISA – Results of an international collaborative study, *Vaccine* 35 (2017) 966–971

