RESEARCH ABSTRACT

SWINE HEALTH

Title: Nanodisc-based mucosal DIVA vaccine for PRRS virus – **NPB #18-170**

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Scientific Abstract: This project examined the level of protective immunity attained in weaner pigs (23±2 days of age) vaccinated either intranasally (IN) or intramuscularly (IM) with a nanodisc (ND)-based vaccine displaying PRRS virus envelope proteins (P-ND). The efficacy of this biologic was compared to that of an in-house inactivated whole virus (IWV) vaccine that was prepared using the same virus (G16X) used as the source of the viral proteins to assemble the P-NDs. For IN immunization the P-NDs were adjuvanted with a whole cell lysate of the *Mycobacterium smegmatis*. For IM immunization, the P-NDs were mixed with the adjuvant Montanide ISA 201 VG (Seppic). As a negative control, a pig cohort received NDs that were void of viral proteins, i.e. empty NDs (E-NDs), which were delivered to the same pigs via both routes (IN/IM) and adjuvanted equally as the P-NDs according to their route of delivery. These four groups (each group n=8) received a booster immunization with the same respective biologic twice at a 25-day interval. To further assess the level of vaccine efficacy, an additional pig cohort (n=5) was immunized once, at the same time as the other cohorts received their first immunization, with a commercial modified live virus (MLV) vaccine. A strict control cohort consisted of pigs (n=3) that were neither vaccinated nor challenged. Forty days after the first immunization, all of the pigs in the trial were challenged IN with virulent virus strain 16244B which, based on the amino acid sequence of GP5, is <97% homologous to G16X. The level of protection stimulated by the vaccines was assessed using objective and subjective parameters indicative of protective immunity including: viremia, gross lung pathology, peripheral blood oxygen saturation (SpO_2), and weight gain. As compared to the non-vaccinated and unchallenged pigs in the strict control cohort, animals that received the E-NDs and were challenged with the virulent 16244B virus as a group exhibited: a three-fold decrease in their rate of weight gain; a significant level of lung dysfunction reflected by hypoxemia (oxygen saturation level of <90%); a significant area of the lung with gross lung pathology (45±9); and a sustained level of viremia (>3 $\log_{10}$

$TCID_{50}$/ml of serum) for the entire period of monitoring (12 days). Pigs in the group that received the P-NDs IN were not protected from the virus challenge, as they exhibited the same extent of adverse events as those observed in the E-ND group. On the other hand, pigs in the cohort immunized IM with the P-NDs exhibited a statistically significant ($p<0.05$) improvement in all of the protective immunity parameters measured, which were not significantly different from the level of protective immunity afforded to pigs immunized with either the IWV or the MLV vaccine, with only one exception. Specifically, all three vaccines delivered IM, namely P-ND, IWV, and MLV, significantly improved the rate of weight gain after the virus challenge, eliminated the presence of hypoxemia, and substantially reduced the extent of gross lung pathology. The one exception consisted of the ability of the MLV to elicit protective immunity capable of terminating the viremia within 10 days after the virus challenge in every pig in this cohort. Nonetheless, the viremia in the cohort of pigs that received the IM P-ND vaccine was extinguished within 12 days after the challenge in 3 out of 8 pigs, with the other 5 pigs exhibiting a clear trend towards an impending viral elimination. Notably, there was no statistical difference in the rate of viremia extinction between the pigs immunized IM with the P-ND or with the IWV vaccine. The lack of protective immunity afforded by the P-ND biologic delivered IN was most likely due to a lack of sufficient amount of viral antigen in the vaccine. The level of protective immunity attained by the P-ND vaccine delivered IM based solely on viral envelope proteins incorporated in their natural configuration into NDs was impressive.