

# A GLP Toxicity and Immunogenicity Study of NB-1008, an Intranasal W<sub>80</sub>5EC-Adjuvanted-Fluzone® Vaccine

T. Hamouda, S. Gracon and J.R. Baker Jr.  
NanoBio Corp., Ann Arbor, MI, USA

Presented at 49<sup>th</sup> ICAAC Annual Meeting  
September 12 - 15, 2009  
San Francisco, CA

For additional information contact:  
John Coffey, Jr.  
Phone: (734) 302-9107  
E-mail: john.coffey@nanobio.com

## ABSTRACT

**Background:** Nanoemulsions (NE) are composed of high-energy nanometer-sized oil-in-water droplets stabilized by surfactants. Previous studies demonstrated that nanoemulsion adjuvanted vaccines are immunogenic when administered intranasally in animal models. In this IND-enabling study, NB-1008 vaccine was tested for potential toxicity and immunogenicity following intranasal (IN) administration in rabbits.

**Methods:** One hundred and thirty New Zealand White rabbits were immunized IN with Fluzone® (2008-2009), phosphate buffered saline (PBS), W<sub>80</sub>5EC-adjuvant, or Fluzone® (15 or 30µg total HA) mixed with 10% or 20% W<sub>80</sub>5EC-adjuvant. Rabbits received 2 doses on study days (SD) 1 and 15. Safety assessments included clinical observations, ophthalmoscopy, body weights and food consumption, body temperatures, serum chemistry, hematology, coagulation and urinalysis, organ weights and organ weight ratios, gross and microscopic pathology. Several sections of the nasal turbinates including the cribiform plate, olfactory bulb, brain, pituitary, and cranial nerves were examined. The immunogenicity assessment included neutralizing antibodies against all the strains present in Fluzone® 2008-2009 vaccine on SD 1, 16 and 29.

**Results:** Clinical, clinical laboratory, gross and histopathological observations were unremarkable following the administration of NB-1008 vaccine. The maximum administered dose (30µg total HA+20%W<sub>80</sub>5EC) was considered the NOAEL. Robust immune responses were elicited on Day 16 to A/Brisbane (H3N2). Day 29 results showed robust immune responses to all the vaccine strains with 90-100% seroconversion for all groups administered NB-1008. No immune response was detected in the rabbits vaccinated IN with Fluzone® alone or PBS alone.

**Conclusions:** These data support initiation of a Phase 1 single dose intranasal study of NB-1008 in healthy volunteers using total HA doses up to 30 µg and nanoemulsion (W<sub>80</sub>5EC-adjuvant) concentrations up to 20%.

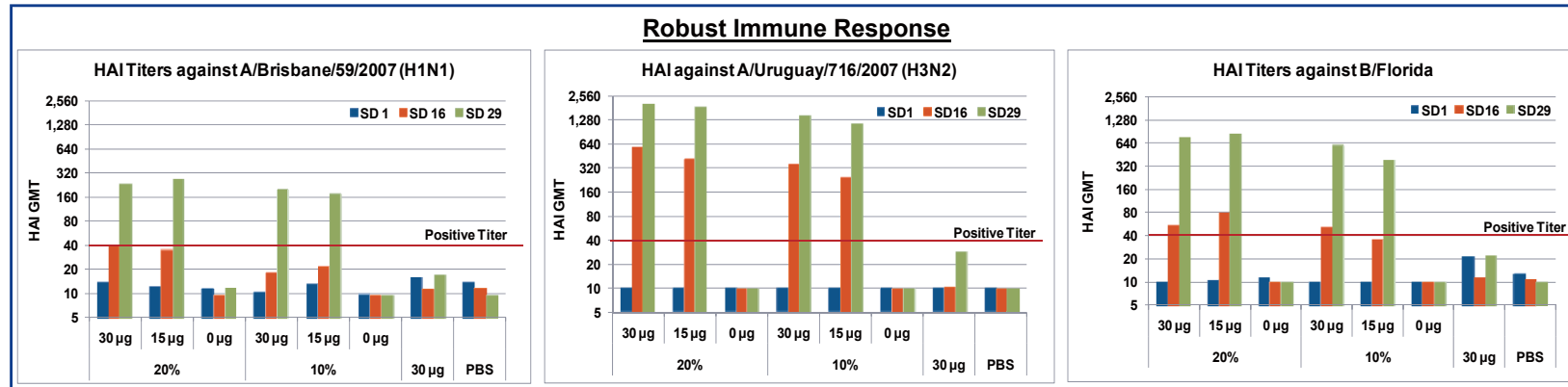
## BACKGROUND

- NanoBio is developing an intranasal influenza vaccine, NB-1008, to increase vaccine efficacy particularly in children and the elderly.
- The vaccine is composed of a nanoemulsion adjuvant (W<sub>80</sub>5EC) mixed with Fluzone® (2008-2009) commercial vaccine.
- The nanoemulsion functions as an adjuvant to enhance the immune response following intranasal (IN) administration without ingredients that have been associated with safety concerns such as bacterial toxins or gene products coding for cytokines (Bell's Palsy – Nasafly, Berna Biotech).
- The nanoemulsion adjuvant mixed with antigen elicits and up-modulates strong humoral and cellular T<sub>H</sub>1-type responses as well as mucosal immunity<sup>1-7</sup> when mixed with different antigens such as recombinant hepatitis B<sup>1</sup>, anthrax protective antigen (PA)<sup>4</sup>, whole vaccinia virus<sup>6</sup> or gp120 protein of Human Immune Deficiency Virus<sup>5</sup>.
- NB-1008 elicited a protective and robust immune responses in naïve ferrets following IN immunization (Poster #G1-877), even with antigen sparing doses.
- The purpose of this study was to determine the potential toxicity and immunogenicity of NB-1008 in rabbits to support human clinical trials with an emphasis on evaluation of cranial nerves and CNS for signs of inflammation.

### References

- Makidon PE, Bielinska AU, Nigavekar SS, et al. (2008). PLoS ONE. 3(8): e2954; 1-15.
- Hamouda T, Chepurinov A, Mank N, et al. (2008). National Foundation for Infectious Disease, 11th Annual Conference on Vaccine Research. Baltimore, MD.
- Myc A, Kukowska-Latallo JF, Bielinska AU, et al. (2003). Vaccine. 21(25-26):3801-14.
- Bielinska AU, Janczak KW, Landers JJ, et al. (2007). Infect Immun. 75(8): 4020-9.
- Bielinska AU, Janczak KW, Landers JJ, et al. (2008). AIDS Research and Human Retroviruses. 24(2): 271-81.
- Bielinska AU, Chepurinov AA, Landers JJ, et al. (2008). Clin. Vaccine Immunol. 15(2): 348-58.
- Warren MR, Becker TJ, Marsh DG, Shelton RS. (1942). J Pharmacol Exp Ther. 74:401-8.

## RESULTS



- | In-Life  | Clinical Laboratories  | Pathology   |
|--|--|---|
| <ul style="list-style-type: none"> <li><b>Behavior</b><br/>No alterations.</li> <li><b>Clinical Observations</b><br/>No significant findings.</li> <li><b>Ophthalmoscopy</b><br/>No significant findings.</li> <li><b>Body Weight and Food Consumption</b><br/>No effect.</li> <li><b>Body Temperature</b><br/>No effect.</li> </ul> | <ul style="list-style-type: none"> <li><b>Serum Chemistry and Urinalysis</b><br/>No effect.</li> <li><b>Hematology</b><br/>Minimal increase in absolute neutrophil counts in male and female rabbits given NB-1008 or 20%W<sub>80</sub>5EC adjuvant alone compared to PBS.</li> <li><b>Coagulation</b><br/>Minimal increase in fibrinogen concentration in female rabbits given NB-1008 or 20%W<sub>80</sub>5EC-adjuvant alone compared to PBS.</li> <li><b>Recovery</b><br/>Absolute neutrophil counts and fibrinogen changes resolved in all groups during the 2-week recovery period and these findings were not considered adverse.</li> </ul> | <ul style="list-style-type: none"> <li><b>Gross Pathology</b><br/>No significant findings.</li> <li><b>Organ Weights and Ratios</b><br/>No significant findings.</li> <li><b>Microscopic Pathology</b><br/>Minimal to mild inflammation of nasal turbinates.<br/>No cranial nerve or CNS inflammation.</li> </ul> |

**Microscopic Pathology**

**Table 2.** Incidence<sup>a</sup> of Treatment-Related Findings in Nasal Turbinates: Terminal Sacrifice

Sex:	Males									Females						
	Fluzone® + 20% W <sub>80</sub> 5EC 30µg	Fluzone® + 20% W <sub>80</sub> 5EC 15µg	20% W <sub>80</sub> 5EC 0µg	Fluzone® + 10% W <sub>80</sub> 5EC 30µg	Fluzone® + 10% W <sub>80</sub> 5EC 15µg	10% W <sub>80</sub> 5EC 0µg	Fluzone® 30µg	PBS 0µg	Fluzone® + 20% W <sub>80</sub> 5EC 30µg	Fluzone® + 20% W <sub>80</sub> 5EC 15µg	20% W <sub>80</sub> 5EC 0µg	Fluzone® + 10% W <sub>80</sub> 5EC 30µg	Fluzone® + 10% W <sub>80</sub> 5EC 15µg	10% W <sub>80</sub> 5EC 0µg	Fluzone® 30µg	PBS 0µg
<b>Acute inflammation, any level<sup>b</sup></b>																
<b>Minimal<sup>c</sup></b>	3/6	2/6	2/3	2/6	1/6	1/3	0/6	0/3	3/6	4/6	1/3	2/6	4/6	0/3	0/6	0/3
<b>Mild</b>	2/6	3/6	1/3	0/6	0/6	0/3	0/6	0/3	1/6	0/6	1/3	2/6	1/6	2/3	0/6	0/3
<b>Infiltrate, neutrophils, lumen, any level<sup>b</sup></b>																
<b>Minimal</b>	4/6	4/6	1/3	3/6	4/6	1/3	0/6	0/3	3/6	2/6	2/3	4/6	2/6	2/3	0/6	0/3
<b>Mild</b>	0/6	1/6	0/3	0/6	0/6	0/3	0/6	0/3	0/6	0/6	1/3	0/6	0/6	0/3	0/6	0/3

<sup>a</sup> Expressed as number of animals with finding/number of animals examined  
<sup>b</sup> Nasal turbinates were examined at 4 levels (cranial, mid, caudal and near cribiform plate). An "any level" finding was created to indicate the highest severity observed at any level of the turbinates.  
<sup>c</sup> Minimal indicated small focal lesion less than 1 mm in width; mild indicated focal or multifocal lesions covering more than 1 mm, although still covering a small portion of the overall turbinate area in the section

## METHODS

**Table 1.** Study Design W<sub>80</sub>5EC-Adjuvanted Fluzone® (2008-2009 Vaccine): A Repeat Intranasal Dose Toxicity and Immunogenicity Study in the New Zealand White Rabbit (Bridge Study #1819-08796)

Treatment	Amount of Influenza Antigen (Total µg HA) <sup>a</sup>	Route	N (F/M)	Number of Doses <sup>b</sup>	Total Volume (250µL/nare)
NB-1008 (Fluzone® + 20%W <sub>80</sub> 5EC)	30	IN	10/10	2	500
NB-1008 (Fluzone® + 20%W <sub>80</sub> 5EC)	15	IN	10/10	2	500
20%W <sub>80</sub> 5EC	0	IN	5/5	2	500
NB-1008 (Fluzone® + 10%W <sub>80</sub> 5EC)	30	IN	10/10	2	500
NB-1008 (Fluzone® + 10%W <sub>80</sub> 5EC)	15	IN	10/10	2	500
10%W <sub>80</sub> 5EC	0	IN	5/5	2	500
Fluzone®	30	IN	10/10	2	500
PBS	0	IN	5/5	2	500

<sup>a</sup> A/Brisbane/59/2007 (H1N1), A/Uruguay/716/2007 (H3N2) and B/Florida/04/2006.  
<sup>b</sup> Doses were administered on Day 1 and 15.  
 Samples for HAI titers were collected on Day 1, 16 and on Day 29.

The vaccine was prepared extemporaneously for each rabbit by mixing the appropriate volume of Fluzone® (2008-2009), PBS and W<sub>80</sub>5EC-adjuvant. The rabbits were anesthetized and placed in a dorsal recumbent position. The required vaccine dose was administered intranasally in a drop wise fashion into each nare using a 1mL syringe and rounded 18 gauge gavage needle. The vaccine was administered on two occasions two weeks apart (Day 1 and Day 15).

## SUMMARY

- NB-1008 elicited robust immune responses in rabbits to all 3 antigens present in the vaccine.
- NB-1008 did not elicit behavioral changes, clinical signs, body weight, food consumption, temperature or ophthalmologic changes.
- Changes in absolute neutrophil count and fibrinogen were not considered adverse.
- Minimal to mild inflammation was observed in the nasal turbinates of NB-1008 and adjuvant only treated rabbits.
- No inflammation was observed in the brain, pituitary, cribiform plate, olfactory bulbs, trigeminal or facial nerves.

## CONCLUSION

- NB-1008, W<sub>80</sub>5EC-adjuvanted Fluzone®, vaccine exhibits safety and efficacy in rabbits.
- NB-1008 is currently being studied in a Phase I clinical trial in 140 subjects in the U.S.