LAWRENCE
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LLNL-TR-666297

# Nanolipoprotein Particles (NLPs) as Versatile Vaccine Platforms for Co-delivery of Multiple Adjuvants with Subunit Antigens from Burkholderia spp. and F. tularensis - Technical Report

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## Disclaimer



This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

| CBM QUARTERLY PROGRESS REPORT | PROPOSAL / CONTRACT NUMBER<br>CBCALL12-PLAT2-2-0010 | |
|---|---|---|
| | PERIOD COVERED BY THIS REPORT | |
| PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR<br>Nicholas O. Fischer, Ph.D. | FROM<br>October 1, 2014 | THROUGH<br>December 31, 2014 |
| APPLICANT ORGANIZATION<br>Lawrence Livermore National Laboratory | | CURRENT TRL<br>TRL - 3 |

TITLE OF PROJECT

Nanolipoprotein particles (NLPs) as versatile vaccine platforms for co-delivery o

DUAL USE RESEARCH OF CONCERN

**Select Agent/Toxin:** Please indicate the select agents or toxins studied in this project.

☐ Does not involve use of virulent select agent, toxin or genetic components

| | | | |
|---|---|---|---|
| ☐ | *Bacillus anthracis* | ☐ | Ebola virus |
| ☒ | *Francisella tularensis* | ☐ | Marburg virus |
| ☐ | *Yersinia pestis* | ☐ | Variola major virus |
| ☒ | *Burkholderia mallei* | ☐ | Variola minor virus |
| ☒ | *Burkholderia pseudomallei* | ☐ | Rinderpest virus |
| ☐ | Botulinum neurotoxin | ☐ | Foot-and-mouth disease virus |
| ☐ | Toxin producing strains of *Clostridum botulinum* | ☐ | Avian Influenza virus (highly pathogenic) |
| | | ☐ | Reconstructed 1918 Influenza virus |

**Experiments of Concern:** The following categories of experiments will be used to determine dual use research of concern. Please indicate the type of research conducted from the list below.

☒ Research conducted does NOT involve any of the listed experiments

| | |
|---|---|
| ☐ | Enhances the harmful consequences of the select agent or toxin |
| ☐ | Disrupts immunity or the effectiveness of an immunization against the select agent or toxin without clinical and/or agricultural justification |
| ☐ | Confers to the select agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that select agent or toxin or facilitate their ability to evade detection methodologies |
| ☐ | Increases the stability, transmissibility, or the ability to disseminate the select agent or toxin |
| ☐ | Alters the host range or tropism of the select agent or toxin |
| ☐ | Enhances the susceptibility of a host population to the select agent or toxin |
| ☐ | Generates or reconstitutes an eradicated or extinct biological agent or toxin from select agent or toxins listed above |

**ABSTRACT:** The goal of this proposal is to demonstrate that colocalization of protein subunit antigens and adjuvants on nanolipoprotein particles (NLPs) can increase the protective efficacy of subunit antigens from *Burkholderia spp*. and *Francisella tularensis* against an aerosol challenge. In the third quarter of the third year, F344 rats vaccinated with adjuvanted NLP formulations were challenged with *F. tularensis* SCHU S4 at Battelle. Preliminary data indicate that up to 65% of females vaccinated intranasally with an NLP-based formulation survived this challenge, compared to only 20% survival of naïve animals. In addition, NLPs were successfully formulated with *Burkholderia* protein antigens. IACUC approval for immunological assessments in BALB/c mice was received and we anticipate that these assessments will begin by March 2015, pending ACURO approval.

## STUDIES AND RESULTS:

***Task 4.4:*** In this Quarter, the in vivo phase of aerosol F. tularensis SCHU S4 challenge experiments in the F344 rat model was completed. In brief, animals were vaccinated three times every 4 weeks with adjuvanted (CpG: ODN 1826) NLPs co-formulated with all three protein antigens (IglC, DnaK, KatG). Animals were vaccinated intranasally (I.N.) or intramuscularly (I.M.). Three unique NLP formulations were tested, varying in amount of adjuvant and antigen material: 5 µg CpG + 10 µg each antigen (5/10), 5 µg CpG + 20 µg each antigen (5/20), and 20 µg CpG + 10 µg each antigen (20/10). In addition, a group was vaccinated 4 weeks prior to challenge with 3 X $10^7$ LVS (I.M.) to serve as a positive control for protective vaccination. A group of unvaccinated naïve animals served as a negative control for protection. The preliminary assessment of the survival data is represented in Figure 1 (Battelle is currently conducting in-depth STATS, QA, and QC). Animals were challenged with 42 ± 6 aerosol $LD_{50}$ of *F. tularensis* SCHU S4. Within two weeks post-challenge, significant differences in the survival rates between females and males were observed. For the females, all NLP-vaccinated groups exhibited enhanced survival compared to the naïve group (Figure 1A). In particular, female animals receiving I.N. administration of 5µg CpG / 20µg antigens (5/20) exhibited >65% survival ($p$ = 0.0093 relative to naïve group). Vaccinated males did not exhibit enhanced survival compared to naïve groups (Figure 1B), and this in turn is reflected in the compiled Male + Female data (Figure 1C). In addition, median survival time was greatly enhanced, particularly in animals receiving I.N. administration of NLP-based vaccine formulations (Figure 1D). Based on our survey of the current literature, these findings represent the first demonstration of significant protection against aerosolized SCHU S4 using purified, recombinant subunit protein antigens. While we cannot currently explain the lack of protection in males we appreciate that addressing this fact is important. However, all published protective efficacy assessments have been conducted using only rats, making it impossible to compare our current survival results of the male groups with previously published data. These findings underscore the need to assess protective efficacy in both male and female test animals.

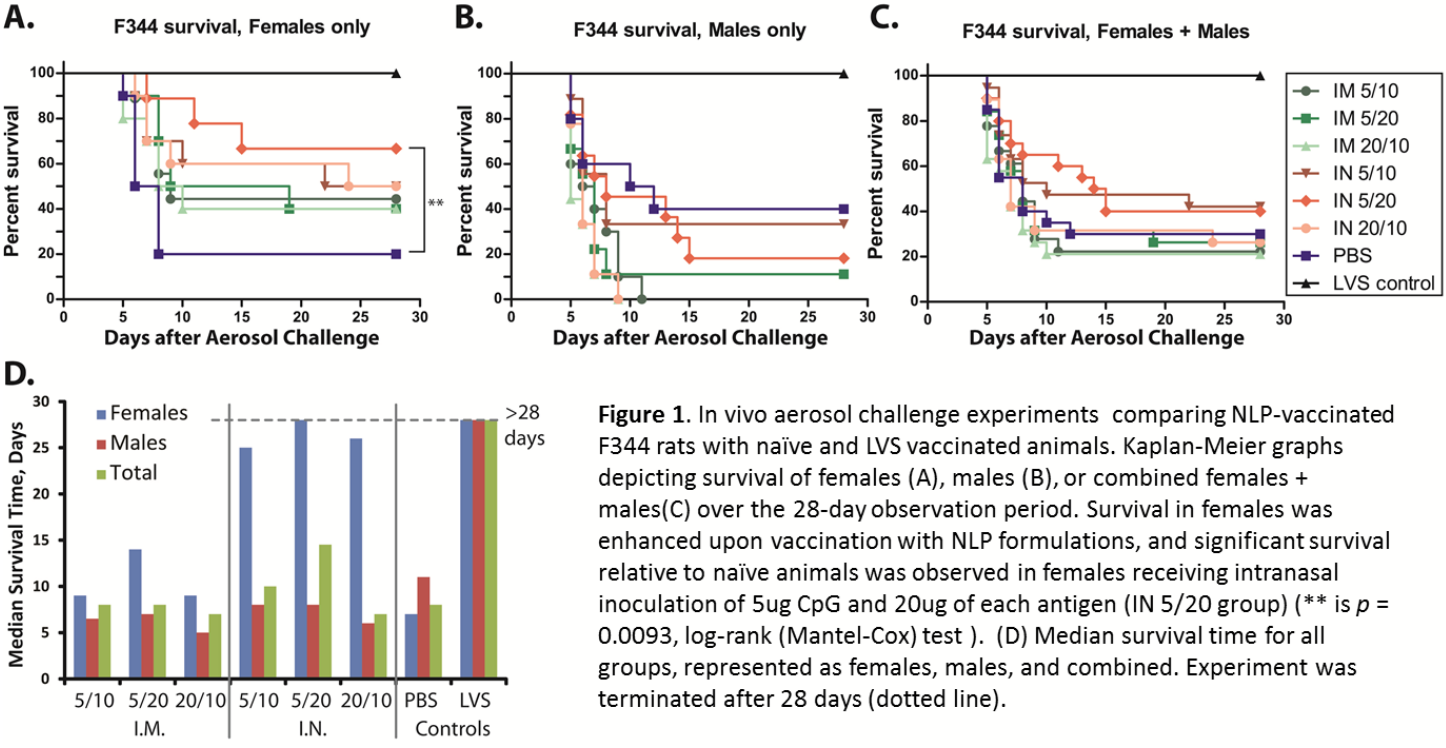


**Figure 1**. In vivo aerosol challenge experiments comparing NLP-vaccinated F344 rats with naïve and LVS vaccinated animals. Kaplan-Meier graphs depicting survival of females (A), males (B), or combined females + males(C) over the 28-day observation period. Survival in females was enhanced upon vaccination with NLP formulations, and significant survival relative to naïve animals was observed in females receiving intranasal inoculation of 5ug CpG and 20ug of each antigen (IN 5/20 group) (** is $p$ = 0.0093, log-rank (Mantel-Cox) test ). (D) Median survival time for all groups, represented as females, males, and combined. Experiment was terminated after 28 days (dotted line).

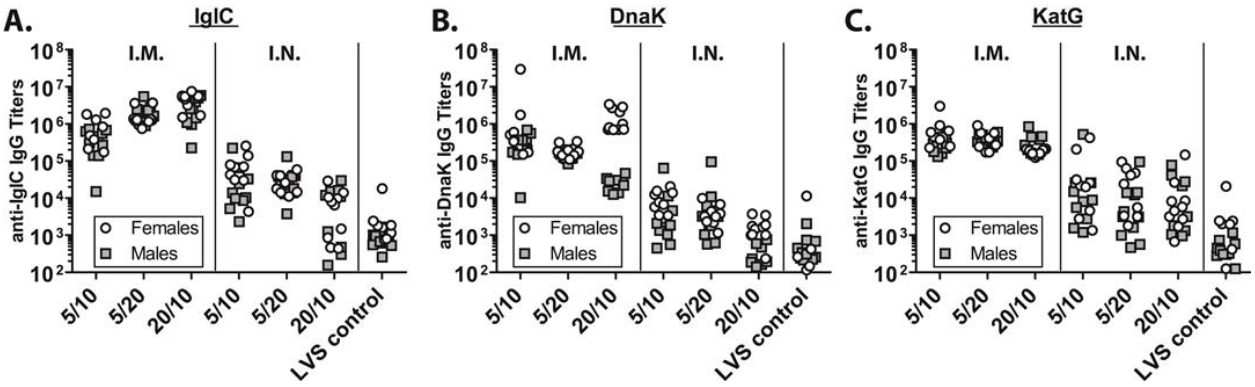


**Figure 2:** Antigen-specific IgG titers in serum were assess in the vaccinated animals one week prior to aerosol challenge. Titers for individual animals were assessed, against each antigen in the vaccine formulation. A) IglC, B) DnaK, C) KatG.

Following the final vaccination at Battelle, individual serum samples were assessed for antigen-specific IgG titers at LLNL (Figure 2). All serum IgG titers in I.M. groups were consistently higher than in corresponding I.N. groups. In addition, IgG titers in I.N. groups ranged widely between individual animals in contrast to I.M. groups. We believe that this variability (and lower overall titers) is not an inherent property of an immune response elicited by I.N. vaccination, but rather an inconsistency in the systematic delivery of the vaccine formulation to the lung via I.N. administration. Recent tests at LLNL have shown that, in contrast to mice, I.N. inoculation in the rat results in greater amounts of inoculum in the stomach, rather than the lungs. Based on these observations, we believe that animals in this study inoculated by I.N. received a significantly lower effective dose of the vaccine formulations. Importantly, even with this lower effective dose, protection via I.N. was observed, leading to the hypothesis that ensuring direct deposition of the NLP inoculum to the lung (*e.g.* intratracheal administration) will afford the protection levels sought by the DoD.

*Task 4.5:* In this Quarter, significant progress has been achieved in preparing novel *Burkholderia* vaccine formulations. In collaboration with Profs. Brett and Burtnick at University of South Alabama (USA), two promising *Burkholderia* protein antigens (Hcp1 and TssM) were conjugated to NLPs (Figure 3). His-tagged protein antigens were readily conjugated to Ni-chelating NLPs, and at least 20 molecules of either protein can be accommodated on a single NLP. Successful conjugation is evidenced by both an increase in absorbance signal intensity at 280nm and a shift to shorter SEC retention times upon increasing loading ratios. A His-tagged variant of *Burkholderia* capsular polysaccharide is currently being prepared at USA. LLNL has received IACUC approval for conducting immunological assessments of NLP formulations incorporating both protein and CPS antigens. Amended documentation will be submitted for ACURO approval shortly.

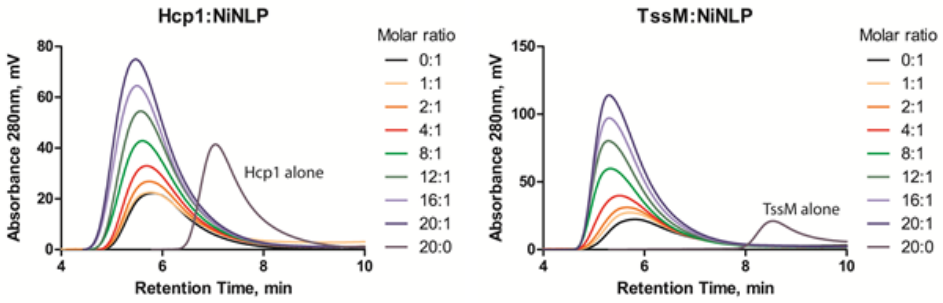


**Figure 3.** Size exclusion chromatography (SEC) was used to assess conjugation of His-tagged Hcp1 and TssM to Ni-chelating NLPs. NLPs were incubated with increasing molar ratios of protein (up to 20:1, antigen:NLP). Absorbance was monitored at 280nm. No unconjugated protein was observed, indicating that all protein was conjugated to the NLP

**ISSUES (TECHNICAL AND PROGRAMMATIC):** No issues were encountered during this Quarter. Amended ACURO documentation will be submitted shortly for immunological assessments of the *Burkholderia* vaccine formulations using a well-established BALB/c mouse model.

**DISCUSSION AND SIGNIFICANCE:** The third Quarter in Year 3 has successfully demonstrated the significant protection of female F344 rats against an aerosolized *F. tularensis* SCHU S4 (42 $LD_{50}$) challenge by vaccinating with protein subunit antigens conjugated to the adjuvanted NLP platform. As indicated in our previous report, intranasal administration elicited a stronger cell mediated immune response than intramuscular administration. We believe that this response, in conjugation with a strong mucosal response, is responsible for the protection observed in the female F344 rats, and can be improved upon to increase the protective efficacy of NLP-based vaccine formulations.

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