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Comparison of lyophilized versus liquid modified vaccinia Ankara (MVA) formulations and subcutaneous versus intradermal routes of administration in healthy vaccinia-naïve subjects

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ABSTRACT

Background: Modified vaccinia Ankara (MVA) is being developed as a safer smallpox vaccine and is being placed in the US Strategic National Stockpile (SNS) as a liquid formulation for subcutaneous (SC) administration at a dose of 1×10^8 $TCID_{50}$ in a volume of 0.5 mL. This study compared the safety and immunogenicity of the standard formulation, dose and route with both a more stable, lyophilized formulation and with an antigen-sparing intradermal (ID) route of administration.
Methods: 524 subjects were randomized to receive either a full dose of Lyophilized-SC, a full dose of Liquid-SC or 20% (2×10^7 $TCID_{50}$ in 0.1 mL) of a full dose Liquid-ID MVA on Days 0 and 28. Safety and immunogenicity were followed through 180 days post second vaccination.
Results: Among the 3 groups, the proportion of subjects with moderate/severe functional local reactions was significantly different ($P = 0.0013$) between the Lyophilized-SC group (30.3%), the Liquid-SC group (13.8%) and Liquid-ID group (22.0%) only after first vaccination; and for moderate/severe measured erythema and/or induration after any vaccination ($P = 0.0001$) between the Lyophilized-SC group (58.2%), the Liquid-SC group (58.1%) and the Liquid-ID group (94.8%) and the reactions lasted longer in the Liquid-ID group. In the ID Group, 36.1% of subjects had mild injection site skin discoloration lasting ≥6 months.

After second vaccination Day (42–208), geometric mean of peak neutralization titers were 87.8, 49.5 and 59.5 for the Lyophilized-SC, Liquid-SC and Liquid-ID groups, respectively, and the maximum number of responders based on peak titer in each group was 142/145 (97.9%), 142/149 (95.3%) and 138/146 (94.5%), respectively. At 180 days after the second vaccination, geometric mean neutralization titers declined to 11.7, 10.2 and 10.4 with only 54.3%, 39.2% and 35.2% of subjects remaining seropositive for the Lyophilized-SC, Liquid-SC and Liquid-ID groups, respectively. Both the Lyophilized-SC and Liquid-ID groups were considered non-inferior (primary objective) to the Liquid-SC group.

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Conclusions: Transitioning to a lyophilized formulation, which has a longer shelf life, will not negatively impact immunogenicity. In a situation where insufficient vaccine is available, ID vaccination could be used, increasing the number of available doses of vaccine in the SNS 5-fold (i.e., from 20 million to 100 million doses).

1. Introduction

Despite the official eradication of smallpox in 1980 [1], the threat of potential emerging zoonotic orthopoxviruses [2] and potential use of variola virus as an agent for biological warfare/terrorism remains a concern. Although cell-culture grown ACAM2000® replaced Dryvax® as the licensed smallpox vaccine in the United States' Strategic National Stockpile (SNS), its safety profile is similar to Dryvax® [3]. IMVAMUNE® is a modified vaccinia Ankara (MVA)-based smallpox vaccine, derived from MVA-597. IMVAMUNE® is replication-restricted to avian cells [4,5] and has an improved safety profile [6–9]; however, its efficacy against variola has not been tested. Both liquid and lyophilized formulations of IMVAMUNE® were tested in clinical trials. The liquid formulation of MVA is currently being added to the SNS and is licensed in Canada and Europe. Due to the need to stockpile IMVAMUNE® for extended periods of time, efforts are underway to transition from the liquid formulation to a lyophilized formulation; stability studies are still ongoing. This study provides a direct comparison of the two formulations to bridge safety and immunogenicity data.

The intradermal (ID) route was historically used for MVA vaccination [10–13] alone, or as a vector for other antigens [14–21], as a means to enhance the immunogenicity of vaccines such as BCG, malaria and HIV, and for antigen sparing effects compared to other routes [22–29]. Though IMVAMUNE® is currently administered subcutaneously (SC), a study with another MVA suggested that ID vaccination can be dose sparing relative to SC and intramuscular routes [28]. Since reducing the vaccination dose would expand the number of doses available in the SNS during an emergency, a formal antigen-sparing assessment of the ID route with IMVAMUNE® was conducted.

2. Methods

2.1. Study design

In this Phase II trial (clinicaltrials.gov NCT00914732), subjects were randomized 1:1:1 to three arms to receive 2 doses of MVA 28 days apart (Fig. 1). The Lyophilized-SC ($n = 165$ subjects) and Liquid-SC ($n = 167$ subjects) groups received 1×10^8 $TCID_{50}$ of vaccine virus in a dose of 0.5 mL reconstituted lyophilate and liquid formulation, respectively, administered subcutaneously into the deltoid area. The Liquid-ID ($n = 191$) group received 2×10^7 $TCID_{50}$ in 0.1 mL per dose [28] in the volar area of the forearm. Staff was blinded to the formulation used for SC administration, but not to ID administration. Immunogenicity was measured using ELISA and Plaque Reduction Neutralization Titer (PRNT) assays.

Note: Many subjects, especially in the Liquid-ID arm had ongoing local (injection site) reactogenicity 28 days after the first vaccination. A protocol amendment allowed subjects to receive the second dose in the contralateral arm if only mild erythema and/or induration from the first vaccination were present at Day 28 (previously subjects were excluded if they had any reactogenicity), to exclude subjects with continued moderate/severe erythema and/or induration from receiving dose 2, and to replace subjects who did not receive dose 2 for any reason.

Subjects reported local and systemic reactogenicity, i.e., solicited adverse events (AE), using a memory aid on Days 0–14 after each vaccination. Unsolicited AEs, i.e., non-reactogenicity events, were collected for 56 days following the initial vaccination. Local reactogenicity symptoms that extended beyond the 15-day memory aid period and were stable for 30 days, and unexpected local reactions, i.e., nodules and discoloration at the vaccination site, were reported as unsolicited AEs. Serious AEs were collected during the entire study. Hematologic and chemistry evaluations were obtained at screening and 14 days after each vaccination. Electrocardiograms and troponin 1 levels were evaluated at screening.

The study was approved by the respective institutional review boards. All subjects provided written informed consent.

2.2. Inclusion criteria

Eligible subjects were healthy, ≥18 years of age and born after 1971, not pregnant, and had an acceptable ECG, a ≤10% risk of myocardial infarction or coronary death using the National Cholesterol Education Program's risk assessment tool, and no evidence of a vaccinia scar, history of smallpox vaccination or history of eczema.

2.3. Vaccine

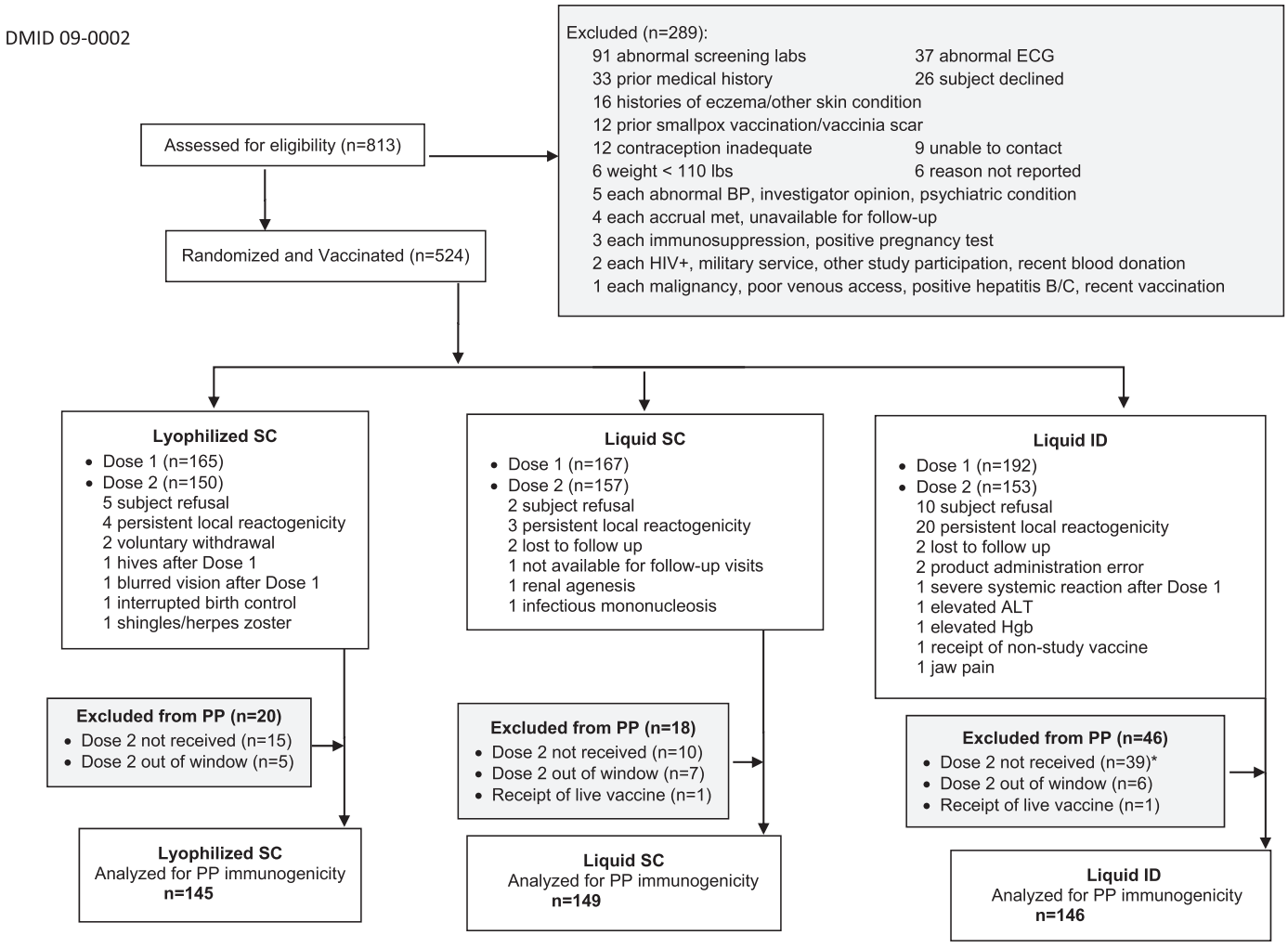
IMVAMUNE® was supplied by Bavarian Nordic (BN) A/S (Kvistgård, Denmark). Liquid-frozen aliquots (2 lots) and lyophilized (freeze-dried) product (1 lot) was supplied as single use vials with an approximate titer of 1×10^8 $TCID_{50}$ IMVAMUNE® per 0.5 mL. Excipients in the lyophilized product included Tris-buffered saline, Tris, NaCl, sucrose, Dextran, and Glutamic acid monopotassium salt monohydrate. Liquid formulation excipients included Tris (hydroxymethyl)-amino methane and NaCl. Sterile water for injection was used for reconstitution of the lyophilized vaccine.

2.4. Immunogenicity assays

The immunogenicity assays were previously described [8,30,31]. BN (primary assays) used vaccinia-Western Reserve (replicating vaccinia) [7] and BN–MVA (non-replicating vaccinia in humans) in the plaque reduction neutralizing antibody ($PRNT_{50}$) assay [8] and ELISA [8], respectively. Saint Louis University (SLU) (secondary assays) used ATCC MVA (VR-1508) as the assay antigen in the ELISA [30] and $PRNT_{60}$ [31] assay. Seroconversion was defined as the ELISA or PRNT value above the cut-off value of the assay. Blood samples were obtained on Days 0, 14, 28, 42, 56 and 208 after first vaccination.

2.5. Statistical analysis

The sample size calculations targeted at least 80% power to test non-inferiority for the primary [geometric mean of peak (GMT) of the BN–$PRNT_{50}$] and secondary (GMT of the BN–ELISA) immunogenicity end points for two investigational arms in reference to a



*Includes one subject who was discontinued after vaccination error at Dose 1, excluded from safety and immunogenicity PP analyses.

Fig. 1. Consort diagram representing the number of subjects assessed for eligibility, randomized to treatment and available for per protocol immunogenicity analysis.

control arm. Assuming a standard deviation of 2.8 for $\log_2$ peak PRNT or ELISA titer in each group (default for BN's $PRNT_{50}$ assay at time of protocol development), a non-inferiority margin of 2.0-fold [32–34], a type I error rate of 1.25%, and drop-out rate of 10%, 165 subjects in each of the three groups were to be enrolled in this study to achieve at least 148 evaluable subjects. To maintain at least 148 evaluable subjects per arm, an additional 29/151 (15.1%) subjects were enrolled to replace subjects who did not receive their second vaccination. Seventeen subjects [Liquid-ID = 10/191 (5.2%), Liquid-SC = 2/167 (1.2%), Lyophilized-SC = 5/165 (3%)] were discontinued due to subject refusal ($P = 0.09$).

The number of subjects with safety events and titers above an assay-specific cut-off and ≥4-fold baseline increase were summarized using frequency, proportion and its 95% two sided exact (Clopper–Pearson) confidence interval. Frequency comparisons between treatment groups were carried out using a Fisher's exact test if large sample approximation criteria were not met. Otherwise a Chi-Square test was applied. The peak PRNT and ELISA titer was calculated as the largest titer obtained for post second vaccination measurements. For each investigational arm, the control arm difference in $\log_2$ GMT and associated two-sided 97.5% CI was used to evaluate non-inferiority (established if upper CI bound <1) and superiority (established if upper CI bound <0).

Except for non-inferiority testing, alpha was not adjusted for multiple testing. Antibody half-life was calculated by fitting a linear regression model to $\log_2$ transformed titers for Days 42, 56, and 208 (negative reciprocal of the slope estimate). The per protocol (PP) population was used for immunogenicity analysis. Subjects excluded from PP ($n = 84$) are detailed Fig. 1.

3. Results

3.1. Study subjects

A total of 524 subjects were enrolled at 8 sites between February 9, 2010 and September 2, 2010. Excluding one vaccination error, 260 males and 263 females were enrolled. Most subjects were non-Hispanic (93.3%) and white (82.0%). The median age was 26.8 years (range: 18–38 years). There were no significant differences in the distribution of gender ($P = 0.54$), ethnicity ($P = 0.06$), race ($P = 0.40$), and age (one-way ANOVA $P = 0.12$) between treatment groups.

3.2. Safety outcomes

All abnormal laboratory values associated with vaccination were considered mild to moderate in severity.

3.2.1. Systemic reactogenicity

Five (3.0%), 0 (0.0%), and 6 (3.1%) of the subjects from Lyophilized-SC, Liquid-SC and Liquid-ID groups, respectively,

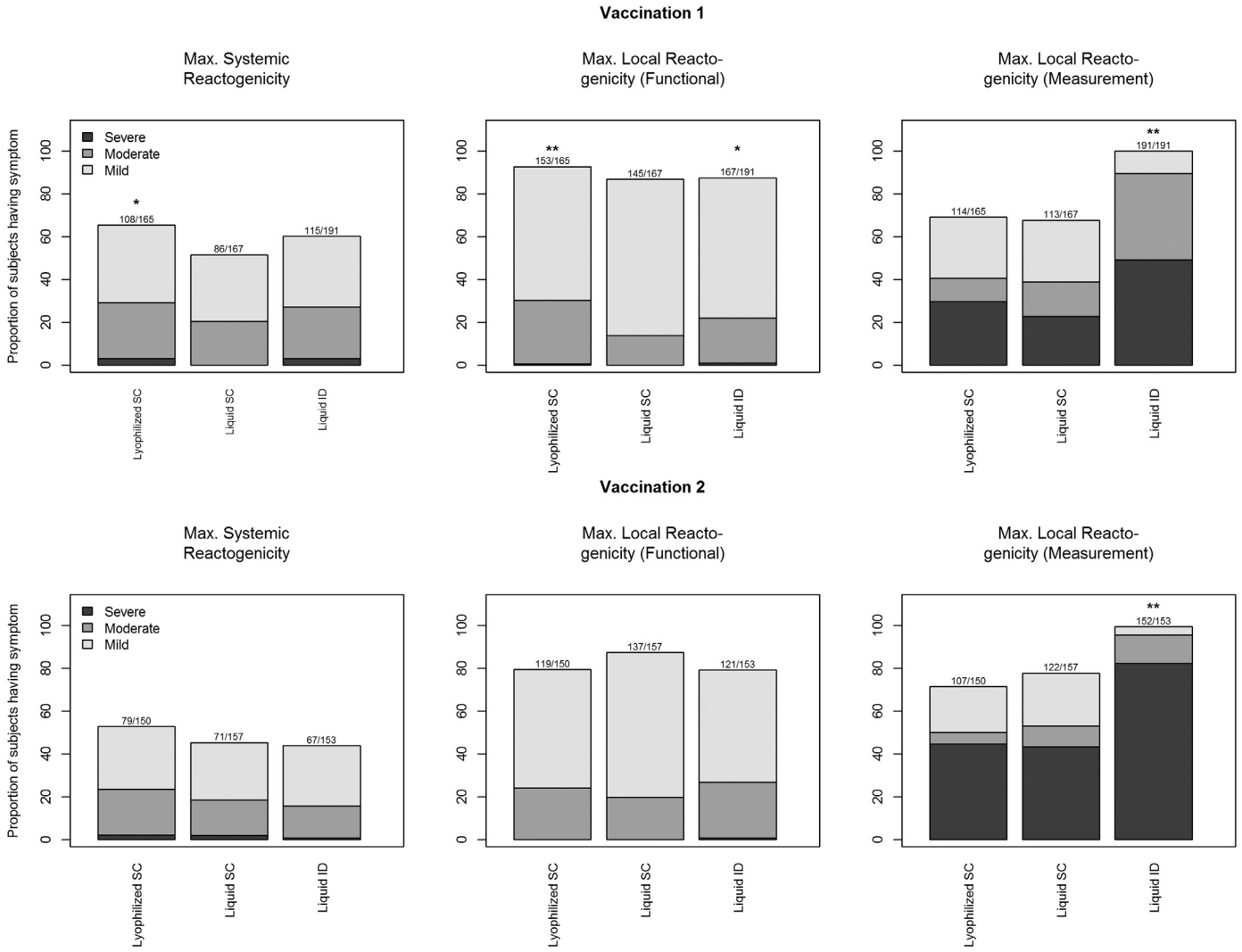


Fig. 2. Maximum severity grade for reactogenicity collected by subjects in the Lyophilized-SC, Liquid SC and Liquid-ID groups for 15 days (Days 0–14) after each vaccination. Systemic reactogenicity events were graded using a functional scale of mild (present but easily tolerated), moderate (able to tolerate routine activity with effort), and severe (unable to continue routine activity). Fever grading scale for oral temperature was mild ≥37.8–<38 °C, moderate ≥38–<39 °C, and severe ≥39 °C; fever is included in the systemic reactogenicity. Local injection site reactogenicity events other than erythema and induration were graded using a functional scale of mild (present but easily tolerated), moderate (able to tolerate routine activity with effort), and severe (unable to continue routine activity). Local injection site erythema and induration were measured and graded as mild (<15 mm), moderate (15–30 mm) or severe (>30 mm). * $P < 0.05$, ** $P < 0.01$.

experienced severe systemic AEs after first vaccination ($P = 0.04$); 3 (2.0%), 3 (1.9%) and 1 (0.7%), respectively, after second vaccination. The rates were significantly higher for both investigational arms after the first vaccination ($P \leq 0.032$) (Fig. 2). After vaccination 1, the proportions of subjects with any systemic reaction were 108/165 (65.5%), 86/167 (51.5%), and 115/191 (60.2%) for the Lyophilized-SC, Liquid-SC, and Liquid-ID groups, respectively ($P = 0.03$ among the three groups). In pair-wise comparisons, the proportion of subjects with any AE for the Lyophilized-SC group was significantly higher than the Liquid-SC group ($P = 0.01$); there was no significant difference between the Liquid-SC and Liquid-ID groups ($P = 0.10$) after first vaccination. After the second or any vaccination, there were no significant differences among the three groups (Fig. 2) or in pairwise comparisons.

When considering severity (Fig. 2) of the systemic reaction (none/mild vs. moderate/severe), there were no significant differences in the proportion of subjects among groups post vaccination 1, 2, or any vaccination. In general, feeling tired was the most common symptom in each group after each vaccination.

One subject each had a moderate fever after first vaccination in the Liquid-SC and Liquid-ID groups, and none after the second vaccination in either group. Two subjects had mild fever after vaccination 1, and 6 subjects had fever (5 mild, 1 moderate) after vaccination 2 in the Lyophilized-SC group.

3.2.2. Local reactogenicity

Four subjects experienced severe local reactions with functional grading (Fig. 2): 1 in the Lyophilized-SC group (pain), and 3 in the Liquid-ID group (itchiness).

The proportions of subjects with moderate/severe functional local reactions post vaccination 1 were 30.3%, 13.8%, 22.0% for the Lyophilized-SC, Liquid-SC, and Liquid-ID groups, respectively ($P = 0.0013$) (Fig. 2); for the comparison among the three groups, $P = 0.0003$ for Lyophilized SC compared to Liquid SC, and $P = 0.04$ for comparison of Liquid ID to Liquid SC). There were no significant differences after vaccination 2 ($P = 0.34$ among groups). However, in pairwise comparisons, the proportion of subjects with moderate/severe local reactions was significantly higher in the Lyophilized-SC group than the Liquid-SC group ($P = 0.02$). Pain at the injection site was the most prevalent local reaction in both of the SC groups and itchiness at the injection site was the most prevalent in the ID group.

Table 1a
BN PRNT per protocol population analysis: summary of number and proportion of responders with titers ≥15, peak geometric mean titers (GMT), and number of subjects with ≥4-fold rise by vaccination and visit.

Study visit day	Group		
	Lyophilized SC Seroconversion, n/N (%) [95% CI] GMT [95% CI] ≥4-fold rise n/N (%) [95% CI]	Liquid SC Seroconversion, n/N (%) [95% CI] GMT [95% CI] ≥4-fold rise n/N (%) [95% CI]	Liquid ID Seroconversion, n/N (%) [95% CI] GMT [95% CI] ≥4-fold rise n/N (%) [95% CI]
Day 0[a]	0/145 (0.0) [0.0, 2.5] 7.5 [,] NA	2/149 (1.3) [0.2, 4.8] 7.7 [7.4, 8.0] NA	2/146 (1.4) [0.2, 4.9] 7.7 [7.4, 7.9] NA
Day 14	60/145 (41.4) [33.3, 49.8]* 10.9 [9.9, 12.0]NIE 6/145 (4.1) [1.5, 8.8]	44/149 (29.5) [22.3, 37.5] 10.0 [9.0, 11.1] 3/149 (2.0) [0.4, 5.8]	56/146 (38.4) [30.4, 46.8] 10.3 [9.3, 11.3]NIE 2/146 (1.4) [0.2, 4.9]
Day 28[b]	61/145 (42.1) [33.9, 50.5]** 10.8 [9.9, 11.9]NIE 6/145 (4.1) [1.5, 8.8]	39/149 (26.2) [19.3, 34.0] 9.6 [8.7, 10.6] 3/149 (2.0) [0.4, 5.8]	68/146 (46.6) [38.3, 55.0]*** 10.8 [9.9, 11.9]NIE 2/146 (1.4) [0.2, 4.9]
Day 42	137/145 (94.5) [89.4, 97.6] 77.6 [62.3, 96.7]NIE 100/145 (69.0) [60.8, 76.4]	137/148 (92.6) [87.1, 96.2] 45.2 [36.4, 56.2] 70/148 (47.3) [39.0, 55.7]	134/146 (91.8) [86.1, 95.7] 54.4 [43.7, 67.8]NIE 82/146 (56.2) [47.7, 64.4]
Day 56	132/144 (91.7) [85.9, 95.6]** 39.4 [31.9, 48.6]NIE 63/144 (43.8) [35.5, 52.3]	117/148 (79.1) [71.6, 85.3] 23.4 [19.4, 28.3] 37/148 (25.0) [18.3, 32.8]	124/146 (84.9) [78.1, 90.3] 33.4 [27.2, 41.0]NIE 59/146 (40.4) [32.4, 48.8]
Day 208	75/138 (54.3) [45.7, 62.8]* 11.7 [10.7, 12.8]NIE 5/138 (3.6) [1.2, 8.3]	56/143 (39.2) [31.1, 47.7] 10.2 [9.4, 11.0] 1/143 (0.7) [0.0, 3.8]	50/142 (35.2) [27.4, 43.7] 10.4 [9.4, 11.5]NIE 5/142 (3.5) [1.2, 8.0]
Peak post vaccination 2	142/145 (97.9) [94.1, 99.6] 87.8 [71.2, 108.3]NIE 105/145 (72.4) [64.4, 79.5]	142/149 (95.3) [90.6, 98.1] 49.5 [40.0, 61.3] 75/149 (50.3) [42.0, 58.6]	138/146 (94.5) [89.5, 97.6] 59.6 [48.1, 74.0]NIE 86/146 (58.9) [50.5, 67.0]
Half Life [days][c]	69	92	77

NIE: non-inferiority established. PRNT titers ≥15 and <75 were designated a titer of 15 by BN. Titer values of <15 (below limit of detection) were replaced by 7.5 (half the lower limit of detection) for analysis. Seroconversion was defined as PRNT value ≥15.

[a] First vaccination.
[b] Second vaccination.
[c] Based on Day 42, 56, and 208. Accuracy of these 3-point estimates was compromised as many Day 208 observations for BN-PRNT were found below the lower limit of detection.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Following any vaccination, the proportion of subjects with any measured erythema or induration at the injection site differed significantly among groups ($P < 0.0001$): the proportions were 79.4%, 84.4%, and 100% for the Lyophilized-SC, Liquid-SC, and the Liquid-ID dose groups, respectively (Fig. 2). Of these, 58.2%, 58.1% and 94.8% had severe local reactions (>30 mm), respectively.

Local reactogenicity lasting at least 30 days, unexpected nodules and skin discoloration at the vaccination site, accounted for 389 (80%) of the unsolicited adverse events reported as associated to vaccination and included 50/165 (30.3%), 42/167 (25.1%), and 128/191 (67.0%) for the Lyophilized-SC, Liquid-SC, and Liquid-ID groups, respectively. There was no significant difference between the Liquid-SC and Lyophilized-SC group ($P = 0.33$). The proportion for the Liquid-ID group was significantly higher than the Liquid-SC group ($P < 0.0001$).

3.2.3. Protocol amendment

A total of 15, 10 and 39 subjects in the Lyophilized-SC, Liquid-SC, and the Liquid-ID dose groups, respectively (Fig. 1) did not receive second vaccination for any reason. Of these, 4, 3, and 20 subjects in the Lyophilized-SC, Liquid-SC, and the Liquid-ID dose groups, respectively, (Fig. 1) did not receive a second vaccination due to persistent local reactogenicity (mild or greater) at the time of second vaccination. To ensure 148 evaluable subjects per arm, a protocol amendment allowed subjects to receive the second dose in the contralateral arm if only mild erythema and/or induration from the first vaccination was present at Day 28. At the time of the writing of the amendment 152 subjects had been enrolled and no subject had severe local reactogenicity at Visit 3 (Days 13–15) or Visit 4 (Days 26–30).

3.2.4. Unsolicited AEs

Overall, 830 unsolicited AEs within 28 days after each vaccination were reported by 356 (68.1%) subjects. Of these events, 486 (58.6%) were considered associated with vaccine and were experienced by 256 subjects. Sixty-four subjects (38.8%) in the Lyophilized-SC group, 57 (34.1%) in the Liquid-SC group, and 135 (70.1%) in the Liquid-ID group experienced an associated adverse event; none of which were severe. The difference among the three groups was significant ($P < 0.0001$). The Liquid-ID group had a significantly higher proportion than the Liquid-SC group ($P < 0.0001$); the difference between the two SC groups was not significant.

One subject in the Lyophilized-SC group reported intermittent moderate chest pain 3 days post vaccination 2. The next day the subject was asymptomatic and had a normal CK, CKMB, Troponin I and ECG.

Four subjects had a serious adverse event (back pain, ischemic colitis, appendicitis and colitis) which were considered not associated with vaccine. All received both vaccinations.

3.3. Immunogenicity

3.3.1. PRNT

BN–$PRNT_{50}$ (primary assay): There was no significant difference in the number (range 0–2) of subjects with a positive titer (≥15) in each group prior to vaccination 1.

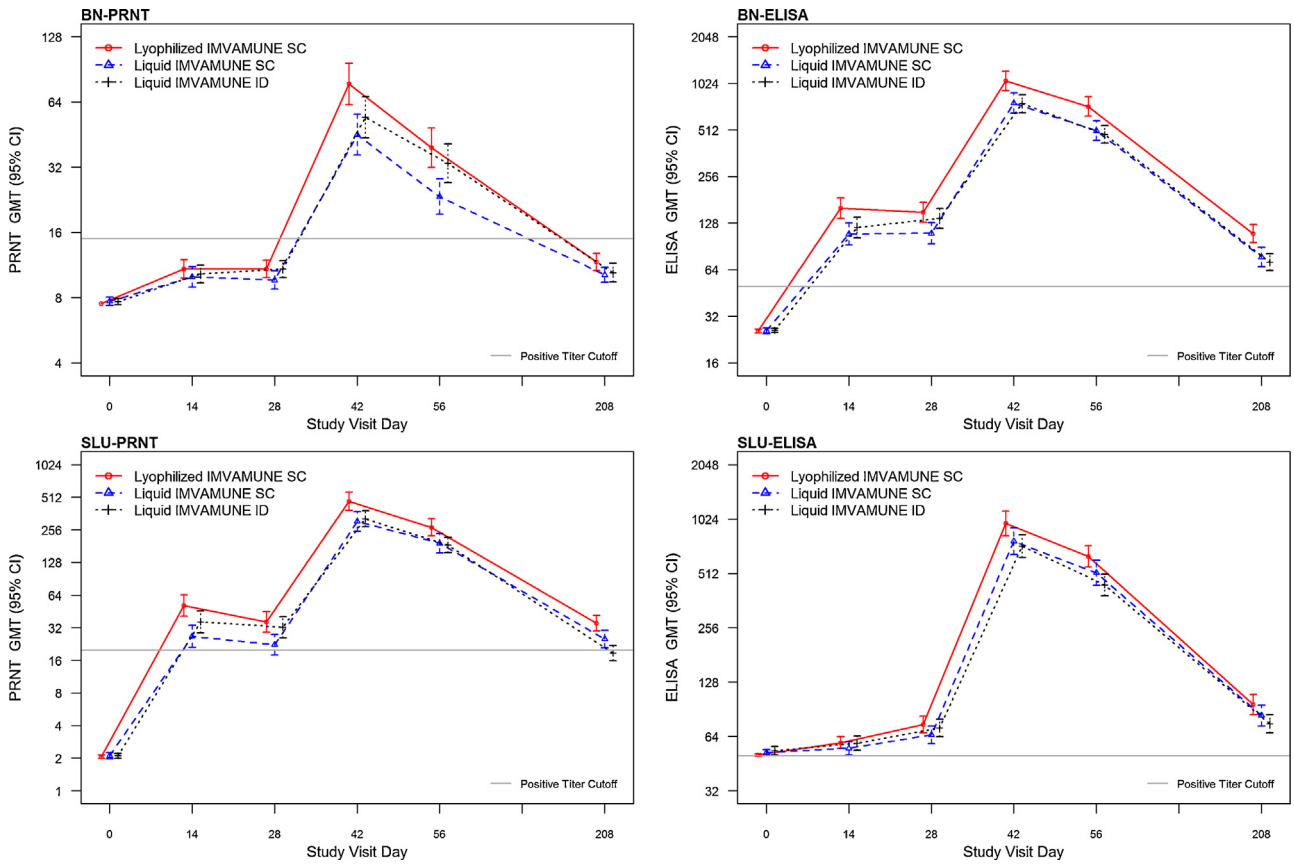


Fig. 3. Per protocol Analysis of Geometric Mean Titers (GMT) and 95% Confidence Intervals (CI) by Group and Day Post First Vaccination for (a) BN PRNT, (b) SLU PRNT, (c) BN ELISA and (e) SLU ELISA. PRNT = Plaque reduction neutralizing antibody. ELISA = Enzyme linked immunosorbent assay.

After second vaccination (day 42–208), GMTs (based on peak titer) were 87.8, 49.5 and 59.6 for the Lyophilized-SC, Liquid-SC and Liquid-ID groups, respectively (Table 1a, Fig. 3) and the maximum number of responders (based on peak titer) in each group was 142/145/(97.9%), 142/149 (95.3%) and 138/146 (94.5%), respectively. The 97.5% CI for the $\log_2$ mean difference between the Liquid-SC and Lyophilized-SC groups and the Liquid-SC and Liquid-ID groups was <1 (Supplementary Table 1). Thus, both the Lyophilized-SC and Liquid-ID groups were considered non-inferior (primary objective) to the Liquid-SC group. For the Lyophilized-SC group, superiority was established (Fig. 4).

The upper limits of the individual 97.5% CIs were <1 for the mean difference between Lyophilized-SC and Liquid-SC groups and for the Liquid-ID group and the Liquid-SC groups for all days (Day 14, 28, 42, 56, 208) individually (exploratory analysis). However, the proportions of responders for the Lyophilized-SC group were significantly larger than the Liquid-SC group on Days 14, 28, 56 and 208 (P: 0.04, 0.0046, 0.028 and 0.01) and the proportions of responders for the Liquid-ID group were significantly larger than the Liquid-SC group on Day 28 only ($P = 0.006$). Per timepoint results are summarized in Table 1a.

SLU–$PRNT_{60}$ (exploratory assay): Although GMTs (based on peak titer) of the SLU–$PRNT_{60}$ were higher compared to the BN–$PRNT_{50}$ (Supplementary Table 2a, Fig. 3), non-inferiority results were similar (Supplementary Table 1, Fig. 4). The maximum number of subjects seroconverting (titer ≥20) in each group after second vaccination was 144/145 (99.3%), 146/149 (98.0%) and 146/146 (100%) for the Lyophilized-SC, Liquid-SC and Liquid-ID groups, respectively. The Lyophilized-SC group also was considered superior to the Liquid-SC group (exploratory analysis).

3.3.2. ELISA

BN–ELISA (secondary assay): There was no significant difference in the number (range 3–6) of subjects with a positive titer (≥50) in each group prior to vaccination (Table 1b). After second vaccination (day 42–208), GMTs (based on peak titer) were 1062.4, 769.3, 757.9 for the Lyophilized-SC, Liquid-SC and Liquid-ID groups, respectively (Table 1b, Fig. 3) and the maximum number of subjects seroconverting (>50) in each group (based on peak titer) after vaccination 2 was 145/145 (100%), 148/149 (99.3%) and 146/146 (100%), respectively. The upper limit of the CI was 1 for the mean difference between the Liquid-SC and Lyophilized-SC group and between the Liquid-SC group and Liquid-ID group (Supplementary Table 1). Thus both the Lyophilized-SC and Liquid-ID groups were considered non-inferior (secondary objective) to the Liquid-SC (Fig. 4). The Lyophilized-SC group was also considered superior to the Liquid-SC group.

Per-visit, exploratory analysis showed that there were no significant differences in GMTs at baseline (Table 1b). The Liquid-ID group was considered non-inferior to the Liquid-SC group while the Lyophilized SC group was not only considered non-inferior but also superior to the Liquid-SC group at each timepoint, respectively (Fig. 4).

However, the proportions of responders in the Lyophilized-SC group (95.2%, 93.5%) were significantly larger than the Liquid-SC group (87.2%, 83.1%) on Days 14 and 208 ($P = 0.02$ and 0.0089) (Table 1b). In the Liquid-ID group, the proportions were significantly larger than the Liquid-SC group on Day 28 ($P = 0.0003$).

The mean $\log_2$ transformed peak titers (positive response >50) were similar for the SLU–ELISA (exploratory assay) results (Supplementary Table 2b). The Lyophilized-SC and Liquid-ID groups

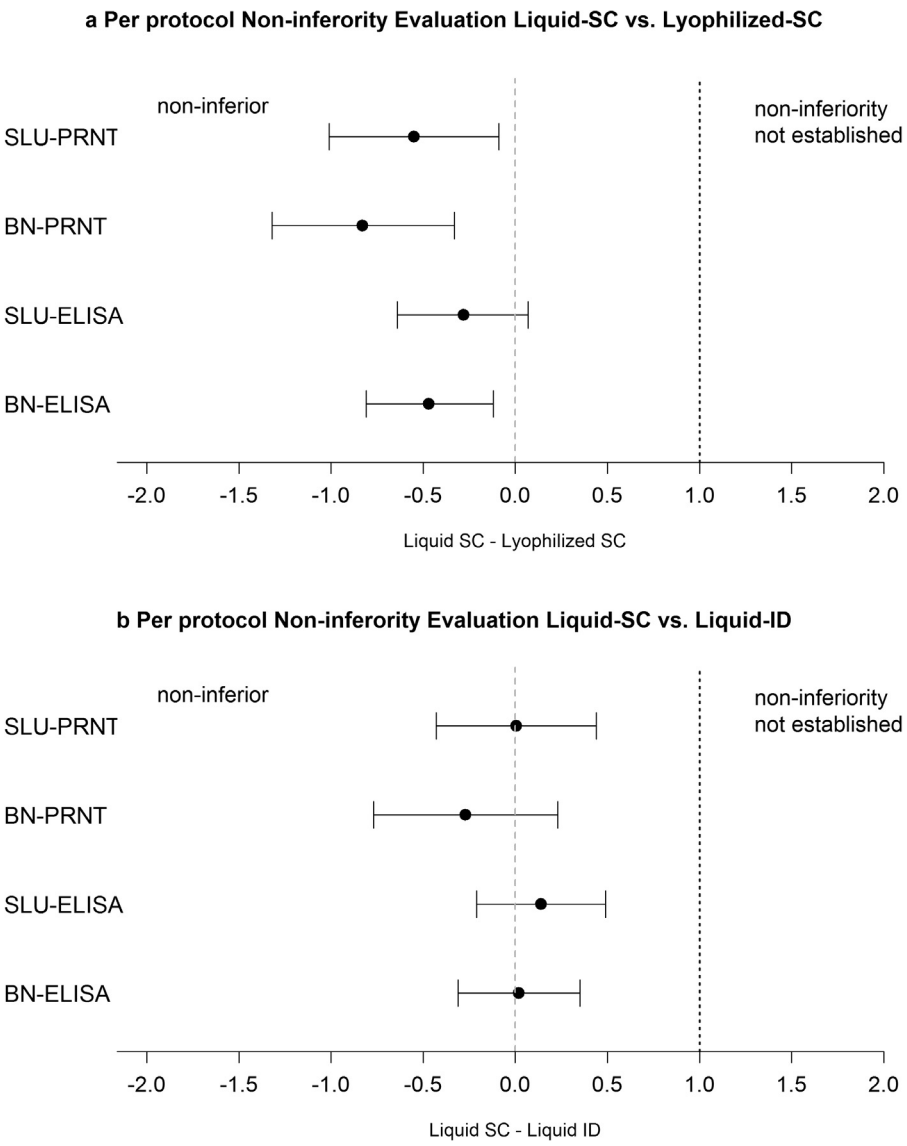


Fig. 4. (a) Per protocol Non-inferiority Evaluation of Liquid-SC vs. Lyophilized-SC 97.5% confidence interval (CI) for the log 2 difference in the geometric means of the peak titers (GMT) for the Liquid-SC and Lyophilized-SC group. The black circles mark the point estimate of the difference and the bars mark the upper and lower limits of the 97.5% CI. The dotted black line marks the non-inferiority margin. The grey dashed line marks a zero difference. If the circle is to the right of the grey dashed line, Liquid SC has higher GMTs than the Lyophilized SC group; if the circle is to the left of the dashed line, Liquid SC has lower GMTs than the Lyophilized SC group. For all assays, the Lyophilized SC group obtained higher GMTs. As the upper bounds of the CIs were below 1 (to the left of the black dotted non-inferiority margin), non-inferiority was established. As the upper bound was also below 0 (grey dashed line) for SLU $PRNT_{60}$, BN $PRNT_{50}$, and BN ELISA, for these assays, the Lyophilized-SC group was superior to the Liquid-SC group. (b). Per protocol Non-inferiority Evaluation of Liquid-SC vs. Liquid-ID 97.5% confidence interval (CI) for the log2 difference in the geometric means of the peak titers (GMT) for the Liquid-SC and Liquid-ID group. As the upper bounds of the CIs were below 1 (to the left of the black dotted non-inferiority margin), non-inferiority was established. Superiority was not established for any assay as the upper bounds of the CIs were not below 0 (grey dashed line).

were considered non-inferior to the Liquid-SC group (Fig. 4). See Supplementary Table 2b for per-visit results.

3.3.3. *Day 42–Day 208 antibody decline*

Antibody concentrations declined rapidly between Days 42 and Day 208 (Fig. 3) with half-life estimates ranging between 42 and 54 days for BN–ELISA, SLU–$PRNT_{60}$, SLU–ELISA, and between 69 and 92 days for BN–$PRNT_{50}$ (Supplementary Fig. 1). Accuracy of these 3-point estimates was compromised for BN–$PRNT_{50}$ and SLU–ELISA assays as many Day 208 observations (>46% and >52%, respectively) were below the lower limit of detection.

4. Discussions

This trial provides the first direct clinical comparison of the liquid and lyophilized IMVAMUNE® formulations. The immunogenicity and safety profiles of both formulations delivered SC were shown to be similar to each other and consistent with prior clinical trial data [8,35]. It is interesting that the proportion of subjects with moderate/severe functional local reactions after the first vaccination was significantly greater in the Lyophilized-SC group compared to the Liquid-SC group. The Lyophilized-SC group also had a superior immune response in three out of the four assays and had higher peak titers and proportion of responders at individual timepoints post vaccination. It is possible that the additional excipients present in the lyophilized formulation increased local reactogenicity and enhanced the immune response. The Liquid-ID arm was immunologically non-inferior to the Liquid-SC arm. The diminished antibody responses 6 months after second dose in all groups are similar to other studies using SC and ID routes [9,28].

As shown with another MVA vaccine [28], the local site reactogenicity was significantly higher after each dose in the ID arm

Table 1b
BN ELISA per protocol population analysis: summary of number and proportion of responders with titers ≥50, peak geometric mean titers (GMT), and number of subjects with ≥4-fold rise by vaccination and visit.

Study visit day	Group		
	Lyophilized SC Seroconversion, n/N (%) 95% CI GMT (95% CI)	Liquid SC Seroconversion, n/N (%) 95% CI GMT (95% CI)	Liquid ID Seroconversion, n/N (%) 95% CI GMT (95% CI)
Day 0[a]	5/145 (3.4) [1.1, 7.9] 25.7 [25.1, 26.4] NA	3/149 (2.0) [0.4, 5.8] 25.8 [24.8, 26.9] NA	6/146 (4.1) [1.5, 8.7] 26.0 [25.1, 26.8] NA
Day 14	138/145 (95.2) [90.3, 98.0]* 159.9 [137.3, 186.1][NIE] 115/145 (79.3) [71.8, 85.6]	130/149 (87.2) [80.8, 92.1] 108.8 [92.4, 128.0] 92/149 (61.7) [53.4, 69.6]	135/146 (92.5) [86.9, 6.2] 119.9 [102.7, 139.9][NIE] 95/146 (65.1) [56.7, 72.8]
Day 28[b]	137/145 (94.5) [89.4, 97.6] 150.4 [129.8, 174.4][NIE] 113/145 (77.9) [70.3, 84.4]	133/149 (89.3) [83.1, 93.7] 110.0 [93.9, 128.8] 93/149 (62.4) [54.1, 70.2]	140/146 (95.9) [91.3, 8.5]* 137.4 [118.6, 159.2][NIE] 107/146 (73.3) [65.3, 80.3]
Day 42	145/145 (100.0) [97.5, 100.0] 1061.9 [920.4, 1225.2][NIE] 145/145 (100.0) [97.5, 100.0]	147/148 (99.3) [96.3, 100.0] 764.6 [657.4, 889.2] 147/148 (99.3) [96.3, 100.0]	146/146 (100.0) [97.5, 100.0] 756.9 [663.4, 863.5][NIE] 146/146 (100.0) [97.5, 100.0]
Day 56	143/143 (100.0) [97.5, 100.0] 725.5 [627.8, 838.4][NIE] 142/143 (99.3) [96.2, 100.0]	147/148 (99.3) [96.3, 100.0] 506.8 [437.6, 586.9] 145/148 (98.0) [94.2, 99.6]	146/146 (100.0) [97.5, 100.0] 478.5 [420.3, 544.7][NIE] 144/146 (98.6) [95.1, 99.8]
Day 208	129/138 (93.5) [88.0, 97.0]** 109.5 [95.8, 125.3][NIE] 91/138 (65.9) [57.4, 73.8]	118/142 (83.1) [75.9, 88.9] 77.4 [66.9, 89.5] 67/142 (47.2) [38.8, 55.7]	123/142 (86.6) [79.9, 91.7] 71.7 [63.3, 81.1][NIE] 57/142 (40.1) [32.0, 48.7]
Peak post vaccination 2	145/145 (100.0) [97.5, 100.0] 1062.4 [920.8, 1225.8][NIE] 145/145 (100.0) [97.5, 100.0]	148/149 (99.3) [96.3, 100.0] 769.3 [661.8, 894.2] 148/149 (99.3) [96.3, 100.0]	146/146 (100.0) [97.5, 100.0] 757.9 [664.4, 864.6][NIE] 146/146 [100.0) (97.5, 100.0]
Half Life [days][c]	53	52	51

NIE: non-inferiority established.
[a] First vaccination.
[b] Second vaccination; titer results below limit of detection (50) are assigned a value of 25. Seroconversion was defined as ELISA value ≥50.
[c] Based on Day 42, 56, and 208.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

compared to the SC arm but not reactogenic enough to limit its use in the event of a smallpox release; all subjects in the Liquid-ID group had at least one solicited local reaction after any vaccination. In addition, local reactogenicity extending beyond the 15 day memory aid period was significantly greater for the ID group than the SC groups. At Day 180, greater than a third of subjects in the ID group continued to have minimal induration or erythema present on exam. The response seen in the ID group was likely due the large number of cells in the dermis that respond to foreign antigen. The finding of nodules and discoloration that was present for extended periods of time was an unexpected outcome not identified in the recent MVA route comparison study, though it has been identified with other vaccines [25,29]. Possible reasons for this difference between the studies include vaccine formulation, vaccine administration technique, or assessment by study staff.

A protocol amendment allowed subjects to receive the second dose of vaccine in the contralateral arm if only none or mild erythema and/or induration from the first vaccination were present and to replace subjects who did not receive dose 2 for any reason. Most of the subjects excluded from receiving second vaccination due to erythema/induration had measurements graded as mild at the time of discontinuation.

Although a vaccinia neutralization titer of 20 to 32 [36–38], depending on the study, is thought by some to be protective against smallpox, a MVA neutralization titer that correlates with protection has not been defined. However, a two-dose regimen of IMVAMUNE® has been shown to provide a similar antibody response as Dryvax® [9], reduced the size of replicating vaccinia takes [9,28] and was as effective if not better than Dryvax® in producing peak 90% variola virus neutralization GMTs [39,40]. The two-dose SC MVA regimen remains the standard against which other regimens/doses are compared until more data are available. Of interest, the half-life, although limited, for the BN neutralizing titers was longer than the half-life of the SLU neutralizing titers. However, the accuracy of the BN half-life was compromised as many of the results at the third time point were below the lower limit of detection. Additional studies are required to more accurately determine half-life.

5. Conclusion

The demonstration of immunogenic non-inferiority (and superiority in 3 of the 4 immunogenicity assays) and safety equivalence of the lyophilized formulation compared to the liquid formulation provides bridging data for the transition from a liquid to a lyophilized formulation of IMVAMUNE®. Additionally, the ID route provided an equivalent immune response to the SC route using 80% less antigen. This antigen sparing effect could significantly increase the number of vaccine doses available in the event of an emergency by 5-fold. Although the ID route had increased local reactogenicity events, the lack of clinical significance of these events makes the route suitable in an emergency situation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.landusepol.2015.07.020.

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