Clinical Study Report

An Open-Label Phase II Study to Evaluate Immunogenicity and Safety of a Single IMVAMUNE® Booster Vaccination Two Years after the Last IMVAMUNE® Vaccination in Former POX-MVA-005 Vaccinees

POX-MVA-023

EUDRACT Number 2007-006297-28

Sponsor	Bavarian Nordic A/S Hejreskovvej 10A DK-3490 Kvistgård
Study vaccine	IMVAMUNE®
Indication	Immunogenicity and safety of a third generation smallpox vaccine IMVAMUNE®
Development Phase	Phase II
Principal Investigator	[redacted]
Date of first screening visit	18 August 2008
Date of last main study visit (Visit 4)	16 December 2008
Date of last follow-up visit	05 June 2009
Coordinating Author	[redacted]
Other contributing authors	[redacted]
Version of the Report	1.0, final
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1 Signatures

I have read this clinical study report and confirm that to the best of my knowledge it accurately describes the conduct and results of the clinical study as outlined in the protocol. The study was performed according to the Good Clinical Practice (GCP) guidelines in operation at the time of the initiation of the study.

2 Synopsis

Name of Sponsor: Bavarian Nordic A/S	Individual Study Table Referring to Part of the Dossier Volume Page	(For National Authority Use only)
Name of Finished Product: IMVAMUNE®		
Name of Active Ingredient: Modified Vaccinia Ankara - Bavarian Nordic (MVA-BN®)		
Title of Study: An open-label Phase II study to evaluate immunogenicity and safety of a single IMVAMUNE® booster vaccination two years after the last IMVAMUNE® vaccination in former POX-MVA-005 vaccinees.		
Principal Investigator: [redacted]		
Study center: [redacted]		
Publication (reference): None at the date of this report		
Clinical development phase: Phase II		
Study period: 18 August 2008 (first subject screened), 05 June 2009 (last subject completed follow-up visit)		
Primary objective: To evaluate the immune response elicited by a booster vaccination with IMVAMUNE® two years after the last vaccination with one or two doses of IMVAMUNE® (i.e. after one- or two-dose priming).		
Secondary objectives: To provide data on safety of IMVAMUNE® from subjects previously vaccinated with one or two doses of IMVAMUNE® and boosted with one IMVAMUNE® vaccination two years after the last IMVAMUNE® vaccination. To compare study Group 1 (two-dose priming = MM) and Group 2 (one-dose priming =MP) with regard to immunogenicity, safety and reactogenicity following the booster vaccination with IMVAMUNE®. To evaluate long-term persistence of anti-vaccinia antibody titers after two years in former POX-MVA-005 study Groups 1, 2 and 4 (Group 4: vaccinia-experienced (vaccinia-primed) and IMVAMUNE® boosted = M-).		
Study design: Open-label, single IMVAMUNE® booster vaccination two years after the last IMVAMUNE® vaccination in trial protocol POX-MVA-005. All subjects from the POX-MVA-005 study who previously received one or two doses of IMVAMUNE® (i.e. study Groups 1, 2 and 4) and completed the POX-MVA-005 study without major protocol violations were the source population for this study. Primarily, the study was designed as a booster study with 75 subjects		

each from Group 1 (MM) and Group 2 (MP) to be enrolled (booster set). Secondary immunogenicity objective of this study was the evaluation of antibody persistence after 2 years including further subjects of these two groups and subjects from Group 4 (M-). These subjects plus the subjects from the booster set form the persistence set.

The source population from the per protocol set of study POX-MVA-005 was as follows:

Group 1 (MM, two-dose primed, N = 168):
Previously vaccinia-naïve subjects having received two subcutaneous (s.c.) vaccinations with 0.5 ml IMVAMUNE® (MM) containing 1 x 10^8 tissue culture infectious dose 50 ($TCID_{50}$) four weeks apart.

Group 2 (MP, one-dose primed, N = 170):
Previously vaccinia-naïve subjects having received first one s.c. vaccination with 0.5 ml IMVAMUNE® containing 1 x 10^8 $TCID_{50}$ and a second s.c. vaccination with placebo (0.5 ml Tris buffer) four weeks apart.

Group 3 (PP, placebo control group, N=164) - not eligible for this study:
Vaccinia-naive subjects having received two s.c. vaccinations with 0.5 ml placebo (Tris buffer).

Group 4 (M-, vaccinia-experienced (vaccinia-primed) & IMVAMUNE® boosted, N = 193):
Previously vaccinia-experienced subjects having received one s.c. booster vaccination with 0.5 ml IMVAMUNE® containing 1 x 10^8 $TCID_{50}$.

The first 75 eligible and consenting subjects per Groups 1 and 2 were planned to be tested on humoral immunogenicity persistence and subsequently receive a booster vaccination with IMVAMUNE® approximately two years (-2 / +3 months) after their last IMVAMUNE® vaccination. After a screening visit, the first 75 eligible subjects should receive a booster vaccination at Visit 1 and should attend further four study visits. All additionally available subjects of Groups 1 and 2, who were not eligible to receive the booster vaccination and all available subjects of Group 4 should have only the screening visit that included a blood draw for persistence testing only.

Number of subjects:
Planned number of subjects to be vaccinated: 150 subjects total, 75 subjects each in Group 1 (MM) and Group 2 (MP).
Actual number of subjects vaccinated ("booster set"): 152 subjects total, 75 subjects in Group 1 (MM) and 77 subjects in Group 2 (MP).
Number of subjects with a blood draw only ("blood draw only"): 152 subjects total, 17 subjects from Group 1, 14 subjects from Group 2 and 121 subjects from Group 4 (M-).
Total number of subjects with persistence data ("persistence set"): 304

Group	Booster set	Blood draw only	Persistence set
Group 1 (MM)	75	17	92
Group 2 (MP)	77	14	91
Group 4 (M-)	0	121	121
total	152	152	304

Main criteria for inclusion:
Groups 1, 2 and 4:
Male and female subjects who participated in study POX-MVA-005 and completed the trial according to protocol.

Test product, dose, route of administration and lot number:
Single s.c. booster vaccination of 0.5 ml IMVAMUNE® containing 1 x 10^8 $TCID_{50}$ MVA-BN® (lot # 0040707).

Duration of treatment:
Subjects received a single vaccination. Study duration: six study visits, up to 36 weeks for each subject.

Reference product:
Not applicable

Criteria for evaluation:

Immunogenicity:

Primary endpoint - peak booster rate:
The peak booster rate is the individual peak response at either Visit 2, 3 or 4 (i.e. nominal week 1, 2 or 4) given as percentage of subjects with either an appearance of antibody titers ≥ 50 in a vaccinia-specific enzyme-linked immunosorbent assay (ELISA) for initially seronegative subjects or an increase of the antibody titer compared to baseline for subjects with a pre-existing antibody titer.

Secondary endpoints:
The booster rate is the percentage of subjects with an appearance of antibody titers ≥ 50 for initially seronegative subjects as determined by ELISA, or an increase of the antibody titer compared to baseline for subjects with a pre-existing antibody titer for each post-baseline study visit/time point.
Kinetics and magnitude of the humoral immune response assessed by ELISA geometric mean titers (GMTs).
Peak booster rate and booster rates for each post-baseline visit given as percentage of subjects with appearance of antibody titers ≥ 6 in a vaccinia-specific plaque reduction neutralization test (PRNT) for initially seronegative subjects or an increase of the antibody titer compared to baseline for subjects with a pre-existing antibody titer.
Kinetics and magnitude of the humoral immune response assessed by PRNT GMTs.
Correlation between antibody titers measured by ELISA and PRNT.
Comparison of antibody persistence 2 years after either one-dose (Group 2; MP) or two-dose (Group 1; MM) priming or after a single booster vaccination in originally vaccinia-experienced (vaccinia-primed) subjects (Group 4; M-).

Safety:
Occurrence of any serious adverse events (SAEs) possibly, probably or definitely related to the study vaccine until the last study visit (Follow-up visit).
Occurrence of unsolicited non-serious adverse events within 28 days after the booster vaccination: Intensity, duration and relationship to vaccination.
Occurrence of any Grade 3 or 4 adverse events (AEs) possibly, probably or definitely related to

the study vaccine within 28 days after the booster vaccination.
Occurrence of solicited local adverse reactions within one week (Days 0-7) after the booster vaccination: Intensity and duration.
Occurrence of solicited general adverse events within one week (Days 0-7) after the booster vaccination: Intensity, duration, and relationship to vaccination.
Further safety parameters: Electrocardiogram (ECG) and cardiac event assessment, vital signs, safety laboratory.

Statistical methods:

Demographic data:
Tests for comparison for the main baseline characteristics were performed for the booster set and the persistence set.

Analysis of safety:
The analysis was based on the safety set. The number of adverse events and number of subjects with at least one adverse event, any related Grade 3 or 4 adverse reaction to the study vaccine within 28 days after the vaccination and the number of subjects with at least one SAE was to be compared between treatment Groups 1 (MM) and 2 (MP). SAEs and adverse events of special interest (SIAEs) i.e. cardiac AEs were listed separately.

Analysis of immunogenicity:
The primary immunogenicity analysis was based on the Full Analysis Set (FAS). For further descriptive purposes, the same statistical procedures were applied to the Per Protocol Set (PPS).

The primary immunogenicity endpoint was the peak booster rate based on the ELISA. The primary hypothesis was that the peak humoral immune response could be re-activated in subjects within each group by a single booster dose of IMVAMUNE® to an observed level of at least 95%. Fisher's exact test was applied to detect any significant differences between Group 1 (MM) and Group 2 (MP).
Additional analyses were performed examining vaccinia-specific ELISA and PRNT booster rates using higher stringency definitions for booster, i.e. twofold and fourfold increase in titers from baseline.
Descriptive statistics and graphical displays were presented as GMT for the ELISA and PRNT of all groups at all visits. In addition, the difference between Group 1 (MM) and Group 2 (MP) GMTs was tested for PRNT and ELISA using the non-parametric Wilcoxon rank test.
Correlations between ELISA and PRNT titers were calculated for each visit.
To assess antibody persistence and the magnitude of the booster reaction a comparison to the visit with the highest response in POX-MVA-005 was performed. The specific data generated in POX-MVA-005 therefore were added to this report for reference.

Summary of results:

Demographic results: Subjects in Group 1 (MM) and Group 2 (MP) were comparable in regard to age, sex, body mass index and ethnic groups, mainly being Caucasian. Since these parameters are also in line with the referring groups of the source population, the two sub-samples are representative for the referring study population of POX-MVA-005. For the

distribution of subjects see table above under “number of subjects” (page 4).

Safety results:

AE	Group 1 (MM) N=75	Group 2 (MP) N=77	Total N=152
SAE any	2	0	2
SAE related	0	0	0
Cardiac AE any (SIAE)	2	1	3
Cardiac AE related (SIAE)	0	0	0
Unsolicited related AE ≥ Grade 3	1	0	1
Solicited general related AE≥ Grade 3	2	1	3
Solicited local AE≥ Grade 3	2	4	6

AEs with a suspected relationship to the study vaccine included mostly AEs in the system organ class (SOC) "General disorders and administration site conditions", with 96.7% of subjects reporting at least one event within this SOC. The most common vaccine-related AEs are listed below.

AE	Group 1 (MM)	Group 2 (MP)	Total
Injection site erythema	80.0%	84.4%	82.2%
Injection site pain	77.3%	83.1%	80.3%
Injection site induration	77.3%	75.3%	76.3%
Injection site swelling	68.0%	62.3%	65.1%
Injection site pruritus	40.0%	46.8%	43.4%
Fatigue	24.0%	31.2%	27.6%
Myalgia	22.7%	23.4%	23.0%
Headache	16.0%	24.7%	20.4%
Nausea	6.7%	14.3%	10.5%

Immunogenicity results:

Booster rates: The primary hypothesis to achieve a peak booster rate of at least 95% determined by vaccinia-specific ELISA was achieved. A single booster vaccination led to peak booster rates of 100% in Groups 1 and 2. Using the more stringent criteria - twofold or fourfold increase of titers - the percentages were still nearly the same. Vaccinia-specific PRNT peak booster rates of 98.7% were achieved.

Frequencies of booster responses in two-dose (Group 1 (MM)) vs one-dose (Group 2 (MP)) primed subjects

Booster assessment		Group 1 (MM) N=75	Group 2 (MP) N=77
ELISA	Peak booster rate	100%	100%
	twofold increase in titer	100%	100%
	fourfold increase in titer	96.0%	100%
PRNT	Peak booster rate	98.7%	98.7%
	twofold increase in titer	98.7%	98.7%
	fourfold increase in titer	98.7%	97.4%

Magnitude of booster responses: ELISA titers rose rapidly following booster vaccination in both study groups. Groups 1 and 2 achieved similar ELISA titers (1822 and 1724, p = 0.665)

given as GMT based on individual peak measures. Compared to the visit with the highest priming response of the two-dose primed Group 1 (MM) (GMT Week 6 = 492) in study POX-MVA-005, the booster response was 3.7 (1822/492) and 3.5 (1724/492) time stronger for Group 1 and Group 2, respectively. For Group 2 (GMT of Week 4 = 59 in study POX-MVA-005), the booster response was 29.2 times (1724/59) the peak of the priming response. Neutralizing antibody titers rose rapidly following revaccination as was seen for GMTs measured by ELISA. PRNT peak GMTs were 166 and 117 (p = 0.113) in Group 1 (MM) and Group 2 (MP), respectively.

Antibody levels (GMT) - persistence, booster and comparison of Group 1 (MM) and Group 2 (MP) to Group 4 (M-) (vaccinia-primed and IMVAMUNE® boosted) – reference to POX-MVA-005

		ELISA			**PRNT**		
***N=**	**Time point**	**Group 1 (MM) 168/92/75**	**Group 2 (MP) 170/91/77**	**Group 4 (M-) 193/121/-**	**Group 1 (MM) 168/92/75**	**Group 2 (MP) 170/91/77**	**Group 4 (M-) 193/121/-**
POX-MVA-005	Week 0	1	1	43	1	1	24
	Week 2	27	29	627	5	5	192
	Week 4	71	59	472	8	7	152
	Week 6	492	41	-	45	6	-
	Week 8	327	23	-	33	4	-
	Week 26	27	5	197	7	2	116
POX-MVA-023	2 years (Baseline)	23	6	135	1	1	10
	Week 1 (V2)	738	490	-	54	29	-
	Week 2 (V3)	1688	1609	-	125	81	-
	peak	1822	1724	-	166	117	-
	Week 4 (V4)	1255	1211	-	64	49	-
	Week 26 (FU)	462	383	-	49	26	-

*N= PPS of POX-MVA-005 / persistence set (two years) / booster set [FAS of POX-MVA-023])

Persistence: 71.7% (66/92) of subjects in Group 1 (MM) and 42.9% (39/ 91) of subjects in Group 2 (MP) were still seropositive two years after the last vaccination. The ELISA GMTs two years after the last vaccination (baseline for this study) were significantly different: 23 in Group 1 (MM) and 6 in Group 2 (MP). The persistence of antibodies two years after priming was low for IMVAMUNE® primed subjects (Groups 1 and 2), but was at a similar level as vaccinia-experienced (vaccinia-primed) subjects at Day 0 (baseline) in POX-MVA-005.

Comparison of the booster response and long-term antibody level after boosting of Group 1 (MM) and Group 2 (MP) back to the original booster response in Group 4 (M-) in POX-MVA-005:
Compared to the booster of Group 4 (M-) at Week 2 in POX-MVA-005 (ELISA GMT = 627), the peak response after booster vaccination in POX-MVA-023 was 2.9 (1822/627) and 2.7 (1724/627) times stronger in Group 1 (MM) and Group 2 (MP), respectively. Six months (Week 26) after the booster immunization the ELISA GMT ratio of Group 1 (MM) and Group 2 (MP) were still 2.3 (462/197) and 1.9 (383/197) fold higher, respectively, compared to Group 4 (M-) (at Week 26 in POX-MVA-005).
PRNT antibody titers measured 26 weeks after the booster vaccination seem lower in recently

primed subjects (Groups 1 and 2 in POX-MVA-023) in contrast to subjects, who were primed a longer time ago (Group 4 (M-) in POX-MVA-005). Two years after priming the PRNT titer dropped to a GMT of 10 in Group 4.
Correlation of ELISA and PRNT: The overall correlation coefficient between the two tests was 0.595 ($p < 0.0001$).

Conclusions:

- All subjects showed a booster response to IMVAMUNE® re-vaccination two years after priming. By this, the primary objective of this study was achieved and the primary hypothesis of at least 95% of subjects showing a booster response was fulfilled.
- Both groups showed a fast and strong booster response indicating a rapid immunological memory response.
- The booster response in subjects after one-dose priming was of similar magnitude as in subjects after a two-dose priming schedule indicating that IMVAMUNE® induces a strong immunological memory even after one-dose priming.
- The ELISA booster response in subjects primed with one or two doses of IMVAMUNE® two years previously was stronger than the booster response in vaccinia-primed subjects. However, the time interval between priming and boosting was much longer in the latter group (>35 years).
- The interpretation of the booster response does not change whether based on ELISA or PRNT. PRNT titers, however, were considerably lower than the ELISA titers. Both tests are correlated ($r = 0.595$).
- The persistence of ELISA antibodies two years after priming was low for IMVAMUNE® primed subjects (Groups 1 and 2), but was at a similar level as vaccinia-primed subjects at Week 0 (baseline) in POX-MVA-005. Six months after boosting, however, the ELISA antibody levels in Groups 1 and 2 were higher than in the vaccinia-primed group (Group 4)
- IMVAMUNE® was safe and well tolerated when administered as a booster vaccination in healthy young subjects. There was no difference whether subjects had received priming vaccination with one or two doses of IMVAMUNE® two years ago.