SYNOPSIS

Name of Sponsor/Company	Janssen Research & Development* Crucell Switzerland AG
Name of Finished Product	Inflexal V
Name of Active Ingredient(s)	Hemagglutinin from influenza strains recommended each season by WHO (Inflexal V)

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Status: Approved

Date: 18 August 2014

Prepared by: Crucell Switzerland AG

Protocol No.: INF-V-A007

Title of Study: A Phase IV, open label study to evaluate the short and long term immune response and cross-protection after vaccination with virosome adjuvant Inflexal® V in elderly subjects

Study Name: CROSSOME study

EudraCT Number: 2011-003188-31

NCT No.: NCT01457027

Clinical Registry No.: NAP

Principal Investigator(s): Prof. Giancarlo Icardi, MD - Dept. of Health Sciences, University of Genoa and Hygiene Unit "San Martino" University Hospital, [redacted] Italy

Study Center(s): [redacted], Italy

Publication (Reference): not applicable

Study Period: 22 November 2011 - 13 August 2012

Phase of Development: IV

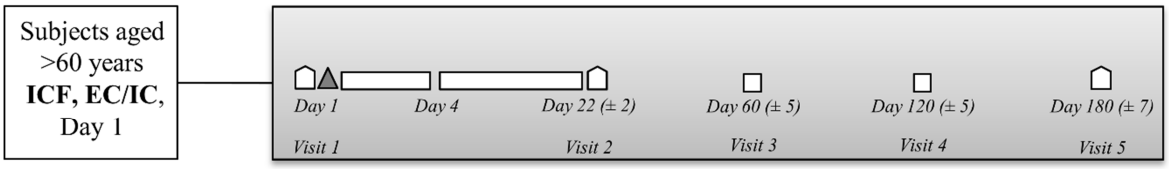
Objectives: ***The primary objective*** of this study was to evaluate the humoral immune response 3 weeks after vaccination with Inflexal V in elderly subjects (aged >60 years) for the 2011/2012 World Health Organization (WHO) recommended 3 seasonal influenza vaccine strains according to the Committee for Medicinal Products for Human Use (CHMP) criteria specified in the guideline "Note for guidance on harmonisation of requirements for influenza vaccines," 1997.

Secondary objectives were the assessment of:

- Immunogenicity parameters 6 months after vaccination for the 3 seasonal influenza vaccine strains
- The cross-protection against 4 selected circulating heterologous A/H1N1 influenza strains 3 weeks after influenza vaccination versus baseline
- The cell-mediated immune response 3 weeks after influenza vaccination versus baseline (for the A/H1N1 vaccine strain and one A/H1N1 heterologous strain) for 30 consecutively enrolled subjects
- The safety and tolerability of the 2011/2012-season influenza vaccine Inflexal V

No formal hypothesis was tested.

Methodology: This was an open label, non-randomized, uncontrolled, single-center study to evaluate the short and long term immune response and cross-protection in elderly adults (aged >60 years) after vaccination with a single intramuscular (IM) dose (0.5 mL) of the virosomal subunit influenza vaccine Inflexal V. A schematic overview of the study is provided below:



ICF: Obtain informed consent; IC/EC: Check inclusion and exclusion criteria.
baseline = Day 1, Visit 1 before vaccination
▲: Single dose IM vaccination (0.5 mL Inflexal V)
⌂: Blood sampling and safety assessment (investigator)
▭: Subject self-assessment of AEs: solicited and unsolicited AEs Day 1 to Day 4 (Subject Diary 1); unsolicited AEs from Day 5 to Day 22 (± 2 days; Subject Diary 2)
□: Safety follow-up (visit or telephone contact)

After written informed consent was obtained and after screening for study entry, blood was drawn from eligible subjects for immunological analysis and the subjects were vaccinated intramuscularly on Day 1 with a single dose of 0.5 mL Inflexal V vaccine. Humoral immune response was planned to be evaluated with the hemagglutiniation inhibition (HI) assay at Day 22±2 days versus baseline and at Day 180±7 days versus baseline for the 3 vaccine strains, and at Day 22±2 days versus baseline for the 4 selected A/H1N1 heterologous strains for which cross-reactivity was planned to be evaluated.

Cross-reactivity was also planned to be evaluated with the microneutralization (MN) assay for the A/H1N1 vaccine strain and the 4 selected A/H1N1 heterologous strains at Day 22±2 days versus baseline. These assessments were limited to the A/H1N1 strain because this strain was expected to continue to circulate and evolve in the near future. Heterologous A/H1N1 strains for cross-reactivity analysis were planned to be selected based on strains which were at the time circulating in the region where the study was carried out.

Cellular immune response was planned to be evaluated for interleukin 2 (IL-2), IL-5, IL-13, and interferon (IFN)-gamma for the A/H1N1 vaccine strain and one A/H1N1 heterologous strain in 30 consecutively enrolled subjects at Day 22±2 days versus baseline.

Each participant received two subject diaries after vaccination. In one diary (Subject Diary 1) they were requested to record all adverse events (AE) (unsolicited and solicited) for 4 days after vaccination (ie, on the day of vaccination and for the 3 days thereafter). In the other diary (Subject Diary 2) they were requested to record all unsolicited AEs from Day 5 to Day 22 (±2 days). Safety assessments were also done by the investigator at baseline and at the subsequent study visits (Day 22±2 days, Day 60±5 days, Day 120±5 days, Day 180±7 days). The overall tolerability of the study vaccine was assessed by both subject and investigator rating.

Number of Subjects (planned and analyzed): A target of 60 subjects were planned to be included in this study. In total, 52 subjects were enrolled and vaccinated with a single dose of 0.5 mL Inflexal V. Because the HI and MN data were invalidated (see **Changes to planned analyses** below), no intention-to-treat and according-to-protocol populations were defined in this study; all analyses have been performed on the safety population. Moreover, no protocol violations determination and classification (major or minor violation leading to exclusion from analysis population) were performed.

No subjects were excluded from the safety analysis.

Diagnosis and Main Criteria for Inclusion: Healthy male and female adult subjects aged >60 years, who had not been vaccinated with a seasonal influenza vaccine for season 2011-2012 and without any known hypersensitivity to any vaccine component were eligible for enrollment into the study. Female subjects had to be confirmed menopausal.

Test Product, Dose and Mode of Administration, Batch No.:

Study vaccine composition

Name	Inflexal V	
Composition	2011-2012-season influenza vaccine (per 0.5 mL dose):	
Active ingredients	HA antigen of A/California/7/2009 (H1N1)-like virus	15 µg
	HA antigen of A/Perth/16/2009 (H3N2)-like virus	15 µg
	HA antigen of B/Brisbane/60/2008-like virus	15 µg
Excipients	Lecithin	117 µg
	Sodium chloride	2.4 mg
	Disodium hydrogen phosphate dihydrate	3.8 mg
	Potassium dihydrogen phosphate	0.7 mg
	Water for injection (ad)	0.5 mL
Dose and frequency	Single dose on Day 1	
Route of administration	IM (*M. deltoideus*)	
Lot No.	3002161	

HA: hemagglutinin

Duration of Treatment: One single standard dose (0.5 ml) of Inflexal V on Day 1.

Changes to planned analyses:

- No interim statistical analysis was performed as the immunogenicity results for the HI and MN assays at Visits 1 and 2 were not available on time.
- Due to quality issues with the HI data generated at the clinical laboratory ([redacted], [redacted] UK), a decision was taken that these HI data could not be used and should be invalidated. In addition, the produced virus titer at [redacted] was too low to setup and perform analysis of the clinical samples in the MN assay. Therefore, no MN data could be generated. The statistical analysis plan (SAP) was adapted accordingly compared to the planned analyses in study protocol.

- The secondary endpoint "Cellular immunity" was modified since only a very limited number of peripheral blood mononuclear cells (PBMCs) were isolated. Only the enzyme-linked immunosorbent spot (ELISPOT) interferon (IFN)-gamma assay was performed with peptide pools of the hemagglutinin (HA)-gene for the 3 homologous strains (no assessments were done for the heterologous strains) and no intracellular staining could be performed.
- The planned subgroup statistical analyses on baseline demographic variables (per baseline HI antibody titers, per previous influenza vaccination status, per age group, per presence of chronic medical conditions) were removed from the SAP.

The primary endpoint of this study could not be evaluated; the only endpoints evaluated for this study were the secondary endpoints "safety and tolerability" and "cellular immunity" (see below).

Criteria for Evaluation:

The ***primary endpoint*** as per SAP was the immunogenicity evaluation for HI antibody titers 3 weeks after vaccination (Day 22±2 days) and was planned to be analyzed according to the CHMP criteria.

- Seroconversion rate, defined as a ≥4-fold increase in HI antibody titer compared to baseline and a titer of ≥1:40 in >30% of subjects.
- Seroprotection rate, defined as an HI antibody titer ≥1:40 in >60% of subjects.
- Geometric mean titer (GMT), defined as >2.0-fold increase in the GMT of HI antibodies.

These primary endpoints were not evaluated (HI data was invalidated).

The ***secondary endpoints*** of the study as per SAP were:

- Immunogenicity: Immunogenicity parameters were planned to be assessed for HI antibody titers for the 3 vaccine strains 6 months after vaccination (Day 180±7 days) according to the CHMP criteria.
- Cross-reactivity: The immune response against the A/H1N1 vaccine strain and 4 selected circulating heterologous A/H1N1 influenza strains was planned to be assessed with the HI assay and the MN assay to measure the neutralizing influenza antibodies 3 weeks after vaccination (Day 22±2 days) versus baseline. An HI titer of ≥1:40 was considered protective. Although the protective titer for the MN assay is not known, to compare these directly with HI, the same criteria as for HI were applied.
- Cellular immunity: Cellular immunity was evaluated for 30 consecutively enrolled subjects. Since only a very limited number of PBMCs were isolated, only the ELISPOT IFN-gamma assay was performed with peptide pools of the HA-gene for 3 homologous strains. The responses 3 weeks after vaccination (Day 22±2 days) were compared with baseline.
- Safety and tolerability: Solicited local and systemic AEs, unsolicited AEs, and tolerability and acceptability.

The "immunogenicity" and "cross-reactivity" secondary endpoints were not evaluated (HI data were invalidated and no MN data were generated).

Statistical Methods:

Baseline was defined as the last assessment performed before vaccination on Day 1.

Demographic data: Baseline demographic variables (gender, age, height, weight, body mass index [BMI], race, ethnicity, smoking status and presence of chronic medical conditions [cardiovascular, respiratory, metabolic diseases]) and subject disposition data were summarized for the Safety population. The planned subgroup analyses on baseline demographic variables were removed from the SAP.

Immunogenicity: All planned statistical analyses related to humoral immunogenicity parameters (HI and MN assayss) were not done.

Regarding cellular immunity, the parameter IFN-gamma (ELISPOT assay) at Day 1 predose and 3 weeks after vaccination (Day 22±2 days) for 30 consecutively enrolled subjects was summarized for each homologous strain using standard descriptive statistics (n, mean, standard deviation (SD), minimum, median, Q1-Q3 and maximum). In addition, the geometric mean and its associated two-sided 95% confidence interval were provided.

Safety: Safety data were summarized descriptively (solicited AEs: up to Day 4; unsolicited AEs: up to Day 22±2 day; serious adverse events [SAE]: up to end of study). The overall frequencies according to intensity were calculated per study day for solicited AEs. In addition, the number and percentages of subjects with at least one solicited local or systemic AE was presented. Frequencies of solicited and unsolicited AEs were presented by System Organ Class (SOC) and preferred term (PT), for all AEs and for vaccination-related AEs.

For body temperature, the number and percentage of subjects with body temperature <38°C or ≥38°C, with number and percentage of subjects for the following categories of temperatures: ≥38.0°C-<38.5°C, ≥38.5°C-<39.0°C, ≥39.0°C-<39.5°C, ≥39.5°C-<40°C, ≥40°C were calculated overall and per day (Day 1 to Day 4). Fever was defined as body temperature ≥38°C.

The number and percentage of subjects was calculated for each tolerability rating and for the acceptability of revaccination, both at Day 22.

Subgroup and interim analyses: No subgroup and interim analyses were finally performed.

RESULTS:

STUDY POPULATION:

This study was conducted between 22 November 2011 and 13 August 2012. In total, 52 subjects were enrolled and vaccinated with 1x0.5 mL Inflexal V. All but one subject completed the study: subject [redacted] (male, aged 69 years) received the study vaccine at Visit 1 but withdrew consent after Visit 4 due to personal reasons not related to the study.

Subject disposition

Subjects, n (%)	Inflexal V 1x0.5 mL N=52
Enrolled	52 (100)
Received study vaccine	52 (100)
Completed study	51 (98.1)
Discontinued study	1 (1.9)

For this study, more female (59.6%) than male (40.4%) subjects were enrolled. The median age was 70.0 years and the majority of subjects were >60 - ≤75 years old (78.8%). Median weight and BMI were 70.00 kg and 25.20 kg/m2, respectively. At baseline, 2 (3.8%) and 8 subjects (15.4%) experienced chronic cardiovascular and chronic metabolic diseases, respectively.

IMMUNOGENICITY:

The only immunogenicity analysis done was the IFN-gamma ELISPOT assay.

Three weeks after study vaccine administration (Day22±2 days, baseline: Day 1), the cellular immune response in the ELISPOT IFN-gamma assay was 10.9 (Day 1: 10.3) spot-forming units per 10^6 PBMCs (SFU/10^6 PBMCs) for strain A/California/7/2009, 12.6 SFU/10^6 PBMCs (Day 1: 20.9) for strain A/Perth/16/2009 and 37.0 SFU/10^6 PBMCs (Day 1: 45.6) for strain B/Brisbane/60/2008. Overall for the 3 vaccine strains, no effect on the IFN-gamma response 3 weeks after vaccine administration was observed.

SAFETY:

Thirty-five subjects (67.3%) in this study reported at least one solicited or unsolicited AE. A total of 24 subjects (46.2%) reported AEs assessed as related to study vaccine, whereof all but one ('headache', 1.9%) were solicited AEs. Note that by definition, all solicited local AEs occurring at the application site were considered related to the study vaccine administration.

Summary: number (%) of subjects with AEs (safety population)

Subjects with at least one AE n (%)	Inflexal V 0.5 mL N=52
AEs (unsolicited and solicited)	35 (67.3)
AEs (unsolicited and solicited, related to study vaccine)	24 (46.2)
Solicited local AEs (vaccine injection site)	24 (46.2)
Pain	23 (44.2)
Erythema	6 (11.5)
Ecchymosis	5 (9.6)
Induration	9 (17.3)
Solicited systemic AE	8 (15.4)
Fever[a]	1 (1.9)
Shivering	4 (7.7)
Malaise	8 (15.4)
Solicited systemic AEs related to vaccine	8 (15.4)
Unsolicited AEs	21 (40.4)
Unsolicited AEs related to vaccine	1 (1.9)
AEs leading to discontinuation	0
SAEs	0

[a] Fever was defined as body temperature ≥38°C.

Serious AE: No deaths or SAEs occurred in the study and no subject discontinued due to AEs.

Solicited local AEs: The most frequent solicited local AE was 'pain', reported for 23 (44.2%) subjects. 'Induration', 'erythema' and 'ecchymosis' were reported for 9 (17.3%), 6 (11.5%) and 5 (9.6%) subjects, respectively.

Most events occurred on Day 1 more than 30 min after vaccination, were of mild or moderate intensity, lasted for 3 or 4 days, and were resolved without sequelae by Day 4. A total of 3 (5.8%) subjects reported the solicited local AE 'pain' of severe intensity. All cases of severe 'pain' lasted 1 day and were resolved without sequelae within 4 days after vaccination. Solicited local AEs were by definition considered related to the vaccine.

Solicited systemic AEs: The most frequent solicited systemic AE was 'malaise', reported for 8 (15.4%) subjects. Fever and 'shivering' were reported for 1 (1.9%) and 4 (7.7%) subjects, respectively.

Most events occurred on Day 1 more than 30 min after vaccination, were of mild intensity, lasted for 3 or 4 days, and were resolved without sequelae by Day 4. No solicited systemic AEs of severe intensity were reported. Subject [redacted] (female, aged 66 years) had fever (38.2 °C) at Day 3, which lasted 1 day. All reported solicited systemic AEs were assessed as related to study vaccine.

Unsolicited AEs: The most common unsolicited AEs were ‘pyrexia’, 'cough' and 'oropharyngeal pain', reported for 8 (15.4%), 7 (13.5%) and 7 (13.5%) subjects, respectively. All cases of ‘pyrexia’ as unsolicited AE were reported at the later stage of the study: after Day 8.

Most events occurred on more than 10 days after vaccination, were of mild intensity, lasted for 3-4 days, and were resolved without sequelae. No unsolicited AEs of severe intensity were reported. Only one unsolicited AE was assessed as related to study vaccine: 'headache' in 1 subject (1.9%). The event lasted for 4 days and was resolved without sequelae. No medication was required to treat this AE.

Vaccine tolerability and acceptance: The tolerability of the study vaccine was rated 'very good' or 'good' by all but 4 subjects (7.7%), who rated 'moderate'. These ratings were similar to the investigator's assessment (rated 'very good' or 'good' for all but 3 subjects [5.8%], for whom they rated moderate). All subjects agreed to be revaccinated with the study vaccine.

CONCLUSION(S):

Due to quality issues with the HI data generated at the clinical laboratory ([redacted], [redacted] UK) a decision was taken that these data could not be used and should be invalidated. Due to the low virus titer produced at [redacted], no MN assay could be set up and no MN data were generated in this study. Since only a very limited number of PBMCs were isolated, only the ELISPOT IFN-gamma assay was performed for the 3 homologous strains (no assessments were done for the heterologous strains) and no intracellular staining could be performed. Therefore, the only endpoints evaluated for this study were the secondary endpoints "safety and tolerability" and "cellular immunity".

Overall for the 3 vaccine strains, no effect on the number of IFN-gamma producing PBMCs 3 weeks after vaccine administration was observed.

The 0.5 ml single dose virosomal influenza vaccine (Inflexal V) administered in elderly subjects aged >60 years was generally safe and well tolerated in this study. There were no new safety findings identified in this study.

SIGNATURES

Signed by	Date	Justification
[redacted]	19Aug2014, 08:25:30 AM, UTC	Document Approval

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