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Safety and immunogenicity of a virus-like particle pandemic influenza A (H1N1) 2009 vaccine: Results from a double-blinded, randomized Phase I clinical trial in healthy Asian volunteers

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ABSTRACT

Methods: A novel, fully bacterially produced recombinant virus-like particle (VLP) based influenza vaccine (gH1-Qbeta) against A/California/07/2009(H1N1) was tested in a double-blind, randomized phase I clinical trial at two clinical sites in Singapore. The trial evaluated the immunogenicity and safety of gH1-Qbeta in the presence or absence of alhydrogel adjuvant. Healthy adult volunteers with no or low pre-existing immunity against A/California/07/2009 (H1N1) were randomized to receive two intramuscular injections 21 days apart, with 100 μg vaccine, containing 42 μg hemagglutinin antigen. Antibody responses were measured before and 21 days after each immunization by hemagglutination inhibition (HAI) assays. The primary endpoint was seroconversion on Day 42, defined as percentage of subjects which reach a HAI titer ≥40 or achieve an at least 4-fold rise in HAI titer (with pre-existing immunity). The co-secondary endpoints were safety and seroconversion on Day 21.

Results: A total of 84 Asian volunteers were enrolled in this study and randomized to receive the adjuvanted ($n = 43$) or the non-adjuvanted ($n = 41$) vaccine. Of those, 43 and 37 respectively (95%) completed the study. There were no deaths or serious adverse events reported during this trial. A total of 535 adverse events occurred during treatment with 49.5% local solicited symptoms, of mostly (76.4%) mild severity. The most common treatment-related systemic symptom was fatigue. The non-adjuvanted vaccine met all primary and secondary endpoints and showed seroconversion in 62.2% and 70.3% of participants respectively on Day 21 and Day 42. While the adjuvanted vaccine showed an increased seroconversion from 25.5% (Day 21) to 51.2% (Day 42), it did not meet the immunogenicity endpoint.

Abbreviations: HAI, hemagglutination inhibition; HA, hemagglutinin; VLP, virus-like particle; RNA, ribonucleic acid; cGMP, current good manufacturing practices; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SE-HPLC, size exclusion high-performance liquid chromatography; MHC, major histocompatibility complex; AE, adverse event; MedDRA, medical dictionary for regulatory authorities.

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Conclusion: In summary, non-adjuvanted gH1-Qbeta showed similar antibody mediated immunogenicity and a comparable safety profile in healthy humans to commercially available vaccines. These results warrant the consideration of this VLP vaccine platform for the vaccination against influenza infection (HSA CTC1300092).

1. Introduction

In June 2009; the World Health Organization declared a pandemic with the emergence of the A/California/04/2009 (H1N1) influenza strain which quickly spread all over the world [1,2]. A functional vaccine produced by traditional methods took 5 months to become available and the total global production was below one billion doses [3]; partly due to the egg-based vaccine production technology with limited yields and time consuming production [4]. The recent development to produce influenza vaccines in mammalian cell culture has removed the full dependence on eggs but limitations remain: the yields are rather low and viruses still need to be processed in a similar time-consuming manner as for the egg-grown vaccines [4]. Advances in molecular biology and recombinant technologies have opened avenues for the design and development of new influenza vaccines which attempt to address these limitations. These technologies include subunit vaccines based on recombinant baculovirus expressed hemagglutinin (HA) in insect cells [5,6]; bacterially produced globular HA domain fused to flagellin [7,8]; nucleic acid based vaccines [9,10]; virosomes (liposomes containing influenza surface antigens) [11] and recombinant virus-like particles (VLPs) produced in plant- or insect cells [12,13]. Meanwhile; with several VLP-based blockbuster vaccines against human papillomavirus and hepatitis on the market; the VLP technology has proven its great benefits [14,15]. The success of these novel technologies is also highlighted by the efforts underway to bring VLP-based influenza vaccines to the market; currently at different stages of clinical development [13,16]. While these approaches hold great promise toward a more rapidly scalable influenza vaccine; most are still reliant on production in eukaryotic cells and cannot approach the yields obtained for recombinant prokaryotic expression systems.

Here we describe the testing of a novel VLP-based influenza vaccine, gH1-Qbeta, produced in *Escherichia coli*. The platform used from Cytos (Schlieren, Switzerland) is based on RNA bacteriophage Qbeta (*Leviviridae*) VLPs and has been shown to be capable of inducing strong antibody responses in clinical trials for therapeutic vaccines [17]. More than 700 subjects have previously been treated with this VLP at doses up to 900 μg. Qbeta coupled to nicotine, angiotensin II or interleukin 1β was used as therapeutic vaccine against nicotine dependence, high ambulatory blood pressure or diabetes, respectively, and displayed good safety and tolerability [17–20]. Each VLP consists of 180 copies of the Qbeta coat protein. These VLPs are highly stable, non-infectious and cannot replicate. Importantly, since humans are not naturally infected by Qbeta, they do not have pre-existing immunity to the VLP. The gH1-Qbeta vaccine tested here consists of the globular head domain (gH1) of hemagglutinin (HA) from the pandemic A/California/07/2009 (H1N1) influenza strain, expressed in *E. coli*, chemically linked to Qbeta VLPs. The resulting conjugated vaccine displays gH1 in a highly ordered and repetitive fashion on the surface of Qbeta VLPs. Single strand RNA (from the recombinant *E. coli* production strain RB791 [21] and therefore without any infectious potential) contained within the VLPs, acts as a built-in adjuvant for the vaccine, shown to promote MHC class II presentation of the antigenic epitopes [22] and at the same time acts as a Toll-like receptor (TLR) 7/8 agonist to boost Th1 responses [23]. The vaccine has been previously described [24] and was shown in pre-clinical studies to protect mice and ferrets from influenza infection and to induce both protective antibodies and, unlike conventional influenza vaccines, potent T-cell responses [25]. Importantly, this vaccine showed excellent cross-protection against heavily drifted strains in mice [24]. This is the first clinical trial with a VLP-based influenza HA vaccine that is produced entirely in bacteria. Qbeta-VLPs can be stockpiled and only the antigen needs to be produced and conjugated to the carrier. Hence, this vaccine could address the shortcomings of current approved vaccines, particularly in cases of an emerging pandemic. The clinical assessment of safety and immunogenicity of gH1-Qbeta is thus an important step toward a proof of concept and here we present its assessment in healthy adult volunteers of Asian origin.

2. Materials and methods

2.1. Vaccine production

The antigen sequence was derived from hemagglutinin of the influenza A virus strain A/California/07/2009 (H1N1), GenBank accession number: ACP41953.1 (amino acids 49-325) and C-terminally extended with a linker sequence (GGGCG) to a total of 281 amino acids. Purification and refolding of gH proteins has been described [24]. The cGMP manufacture of recombinant gH1 was performed in a 100 L fermenter at Biomeva GmbH (Germany) and was formulated to contain a final concentration of 10% glycerol at 1.9 mg/mL, stored at ≤−65 °C.

The cGMP production of the recombinant VLP in *E. coli* RB791 was performed in an 800 L glycerol fed batch at Lonza AG (Switzerland) [26]. Purified Qbeta was stored at 3 mg/mL between −60 °C and −90 °C. To manufacture the drug substance gH1 was cross-linked to Qbeta using succinimyl 6-[(maleimidopropionamido)-hexanoate] and formulated in PBS at a concentration of 1.9 mg/mL containing 0.01% Tween-20. Purity and integrity of the VLP were confirmed by SDS-PAGE and size-exclusion HPLC respectively, for details see Supplemental Material and Methods.

For clinical use gH1-Qbeta (batch 12036) was formulated in 20 mM sodium phosphate, 150 mM sodium chloride, 1.5% (v/v) glycerol, 0.01% (v/v) Tween-20 and water for injection (pH 7.2) and filled and finished by Symbiosis Pharmaceutical Services Ltd. (Scotland, UK). It was supplied in 2 mL single-use vials, filled with 350 μL at a concentration of 0.4 mg/mL (determined by protein content) and stored at ≤−65 °C. The purity and the integrity of the VLP were assessed by scanning densitometry after SDS-PAGE and SE-HPLC, respectively. The coupling density of gH1-Qbeta was determined by SDS-PAGE as 31% and endotoxin levels (according to Ph. Eur.2.9.19) were <0.6 EU/mg protein.

Other components of the vaccine (adjuvant, diluent) were provided in the same 2 mL single use vials. The adjuvant (2% Alhydrogel® EP-grade, Brenntag-Biosector, Denmark) adjusted with diluent (phosphate buffered saline solution containing 0.01% Tween-20 (v/v) and 1.5% (v/v) glycerol, pH 7.2) to a final aluminum concentration of 4 mg/mL with a fill volume of 300 μL, was kept refrigerated (2–8 °C). Diluent vials were filled with 300 μL and stored at −20 °C. Immediately prior to injection the vaccine (250 μL) was mixed with equal volumes of alhydrogel or diluent

in an empty, 2 mL sterile vial provided, and 500 μL were injected in the deltoid muscle using a masked syringe with a 25G, 16 mm needle.

2.2. Study design

This was a double-blinded, 1:1 randomized Phase 1 healthy volunteer study conducted at two sites in Singapore. The study was designed to assess the safety, tolerability and immunogenicity of the vaccine in healthy adults with no or low pre-existing immunity to A/California/07/2009 (H1N1). Subjects received two intramuscular injections, of 100 μg vaccine (42 μg HA) per dose, 21 days apart, either non-adjuvanted or adjuvanted with 2% alhydrogel, in a total volume of 500 μL per injection. A total of 84 subjects were randomized to the two treatment arms. Study personnel and participants were blinded to the treatment allocation, except for the independent statistician from the Singapore Clinical Research Institute (SCRI), generating the randomization list and the unblinded clinical research coordinator, mixing the vaccine with alhydrogel or diluent prior to injection.

2.3. Subjects and study procedures

Study approval was obtained from the Singapore Health Sciences Authority (HSA) and the Centralized Institutional Review Board (CIRB Ref: 2012/906/E) and the study was performed in agreement with the International Conference on Harmonisation guidelines on Good Clinical Practices, laws and regulatory requirements in Singapore and monitored by SCRI. A written informed consent was obtained from each subject prior to screening. Subjects were first enrolled on May 16, 2013 with the last visit on August 2, 2013.

Participants, between 21 and 64 years of age, with satisfactory baseline medical assessment and laboratory values within the normal ranges were eligible. Exclusion criteria were presence of acute infection during 14 days preceding the first vaccination, a temperature ≥38 °C at the date of the first vaccination, and the receipt of immunoglobulins or blood products within 9 months prior to enrolment or during the study. Additional exclusion criteria were receipt of seasonal influenza vaccine in the past 2 years, or any licensed vaccine within 30 days prior to the first injection or HAI titers >1:40 at screening. Concomitant medications (except other vaccines) were not restricted. Women of childbearing potential had to have a negative pregnancy test at each visit.

2.4. Safety assessments

Safety of the vaccine was a co-secondary endpoint, with the following factors assessed: vital signs: (blood pressure, pulse, respiratory rates and body temperature) at all visits (Days 0, 21 and 42) and on vaccination days pre- and 60 min post-vaccination, hematology and blood biochemistry at all visits, solicited and unsolicited local/systemic events (symptom diaries) 21 days following each vaccination. All adverse events (AEs) were coded according to the MedDRA adverse event dictionary (version 12.1) [27] and graded for severity using the FDA guidance document for the toxicity scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials [28].

2.5. Immunogenicity assessments

Screening samples were assessed using standard, non-validated HAI assays at Duke-NUS Graduate Medical School. All further immunogenicity assessments on sera of recruited volunteers from baseline (Day 0), Day 21 and Day 42 were performed on blinded samples, under GLP conditions, using validated HAI assays at Southern Research Institute (Birmingham, AL). In addition to A/California/07/2009 (H1N1) cross-reactive immunogenicity against A/Brisbane/10/10 (H1N1) and A/Georgia/01/13 (H1N1) was tested. All virus strains were purchased from the Centers for Disease Control and Prevention (CDC; Atlanta, GA).

2.6. Statistical methods

An unblinded research coordinator randomly assigned subjects 1:1 to the adjuvanted or the non-adjuvanted group. A computer-generated list (SAS® software, NC, USA) with randomly permutated block sizes of 4 and 6 was provided by SCRI. A sample size of 32 subjects per arm was required to achieve the FDA criterion for seroconversion with a power of 80%, assuming an incidence of 65% [29]. To compensate for 20% drop-outs 40 subjects per arm were planned. The study was not powered to achieve the FDA criterion for seroprotection.

The primary endpoint was seroconversion against A/California/07/2009 (H1N1) by HAI on Day 42, defined as either a pre-vaccination HAI titer <10 and a post vaccination HAI titer ≥40, or a pre-vaccination HAI titer ≥10 and minimum four-fold rise in post-vaccination HAI titer. The co-secondary endpoint (with safety) was seroconversion on Day 21. In addition, geometric mean titers (GMT) and the percentage of subjects achieving seroprotection (HAI titer ≥40), were calculated, the latter only for subjects with baseline HAI titers <40. Geometric mean titer fold rise (GMR) was calculated and GMT and GMR were compared between groups on log-transformed HAI titers using the two-sample *t*-test [30–32]. The 95% CIs of GMT and GMR were constructed by exponential transformation of related 95% CIs based on the log-transformed HAI titer data. Values shown are for the modified Intention-to-treat (ITT) population, not including two subjects that withdrew consent prior to receiving the first dose.

AEs and severe AEs were summarized by treatment group with each subject counted once per AE category with the highest severity of treatment emergent AE (Day 0-Day 42).

3. Results

3.1. Study subjects

Of 156 healthy volunteers consented and screened, 84 were randomized to the treatment groups and scheduled to receive adjuvanted ($n = 43$) or non-adjuvanted ($n = 41$) vaccine. Two subjects from the non-adjuvanted group withdrew consent prior to their first vaccination and two more withdrew consent after they had received the first dose. A total of 43 (adjuvanted) and 37 (non-adjuvanted) subjects completed the study (Fig. 1).

The mean age of enrolled subjects was 39.5 years with 61% of them being male. All were of Asian ethnicity with a Chinese majority (79%, Table 1).

3.2. Safety

Eighty-nine percent of subjects experienced at least one AE during the study (Day 0-Day 42). No serious/life threatening AEs were reported. A total of 11 (13.4%) subjects developed at least one severe AE (grade 3). In total there were 535 AEs reported (278 in the adjuvanted and 257 in the non-adjuvanted arm), of which 265 (49.5%) were local (Table 2). The most frequent local symptoms were pain and muscle ache, followed by limitation of movement in their vaccinated arm and itch (Fig. 2A). The most common treatment-related non-local symptom was fatigue, followed by myalgia, headache, oropharyngeal pain and rhinorrhea (Fig. 2B). Most AEs (76.4%)

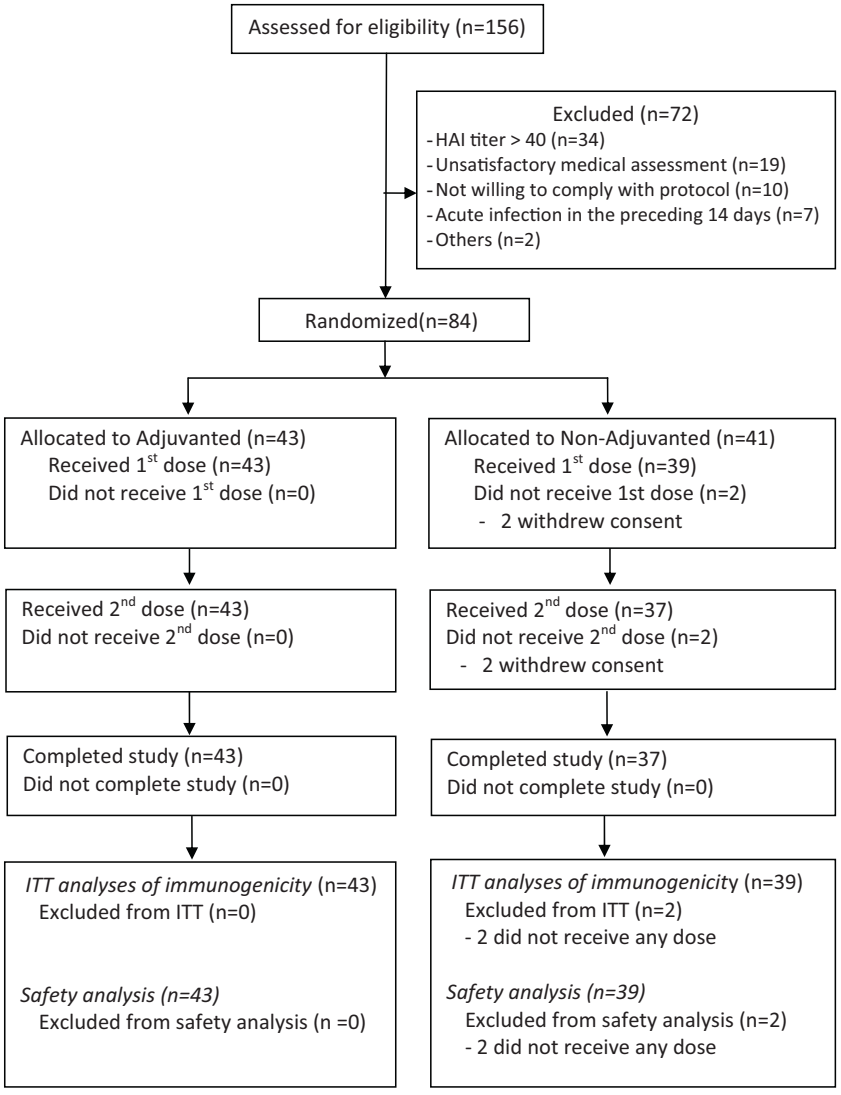

Fig. 1. Subject flow and disposition.

were mild; with 15.3% moderate and 8.2% severe. All AEs were resolved by day 42 except three: cataract in one subject; and two symptoms (tiredness and running nose from allergic rhinitis) in a second subject, considered unrelated to gH1-Qbeta and still ongoing at the last visits. No modification was made to the study drug administration because of any AE. The AEs profile was comparable between the adjuvanted and the non-adjuvanted group (Table 2 and Fig. 2).

3.3. Immunogenicity

The immunogenicity of the vaccine with and without alhydrogel adjuvant was assessed by HAI titers against A/California/7/2009 (H1N1) at Day 42. The proportion of seroconverted subjects after two doses of vaccine is shown in Table 3. In the adjuvanted group, 22/43 (51.2%, 95% CI: 36.8 to 65.4%) and in the non-adjuvanted group 26/37 (70.3%, 95% CI: 54.2 to 82.5%) achieved

Table 1
Demographic characteristics of subjects.

Characteristic	Adjuvanted ($n=43$)	Non-adjuvanted ($n=39$)	Total ($n=82$)
Age (years)	$n=43$	$n=39$	$n=82$
Mean (SD)	37.7 (9.5)	41.6 (10.8)	39.5 (10.3)
Median (IQR)	36.0 (14.0)	42.0 (16.0)	39.0 (13.0)
Min, Max	21, 60	21, 63	21, 63
Gender *n* (%)			
Male	28 (65.1)	22 (56.4)	50 (61.0)
Female	15 (34.9)	17 (43.6)	32 (39.0)
Ethnicity *n* (%)			
Chinese	34 (79.1)	31 (79.5)	65 (79.3)
Malay	5 (11.6)	2 (5.1)	7 (8.5)
Indian	3 (7.0)	4 (10.3)	7 (8.5)
Others[1]	1 (2.3)	2 (5.1)	3 (3.7)

[1] Javanese, Burmese and Pakistani.

Table 2
Summary of adverse events (treated population).

	Number of subjects (%)			Number of events		
	Adjuvanted ($n=43$)	Non-adjuvanted ($n=39$)	Total ($n=82$)	Adjuvanted	Non-adjuvanted	Total
Subjects with at least one AE	38 (88.4)	35 (89.7)	73 (89.0)	NA	NA	NA
Local symptoms	35 (81.4)	35 (89.7)	70 (85.4)	136	129	265
Systemic symptoms	30 (69.8)	25 (64.1)	55 (67.1)	142	128	270
Severity[1]						
Grade 1 (Mild)	38 (88.4)	34 (87.2)	72 (87.8)	226	183	409
Grade 2 (Moderate)	11 (25.6)	14 (35.9)	25 (30.5)	35	47	82
Grade 3 (Severe)	6 (14.0)	5 (12.8)	11 (13.4)	17	27	44
Outcome[2]						
Recovered	38 (88.4)	35 (89.7)	73 (89.0)	276	256	532
Recovered with sequalae	0	0	0	0	0	0
Not recovered	1 (2.3)	1 (2.6)	2 (2.4)	2	1	3
Relationship to IP						
Not related	7 (16.3)	6 (15.4)	13 (15.9)	12	17	29
Unlikely related	12 (27.9)	7 (17.9)	19 (23.2)	41	16	57
Possibly related	20 (46.5)	20 (51.3)	40 (48.8)	73	86	159
Probably related	23 (53.5)	20 (51.3)	43 (52.4)	62	50	112
Definitely related	33 (76.7)	33 (84.6)	66 (80.5)	90	88	178
Treatment given for AE	7 (16.3)	6 (15.4)	13 (15.9)	13	12	25

[1] There was no AE of Grade 4 (potentially life threatening) severity.
[2] There was no AE that lead to death.

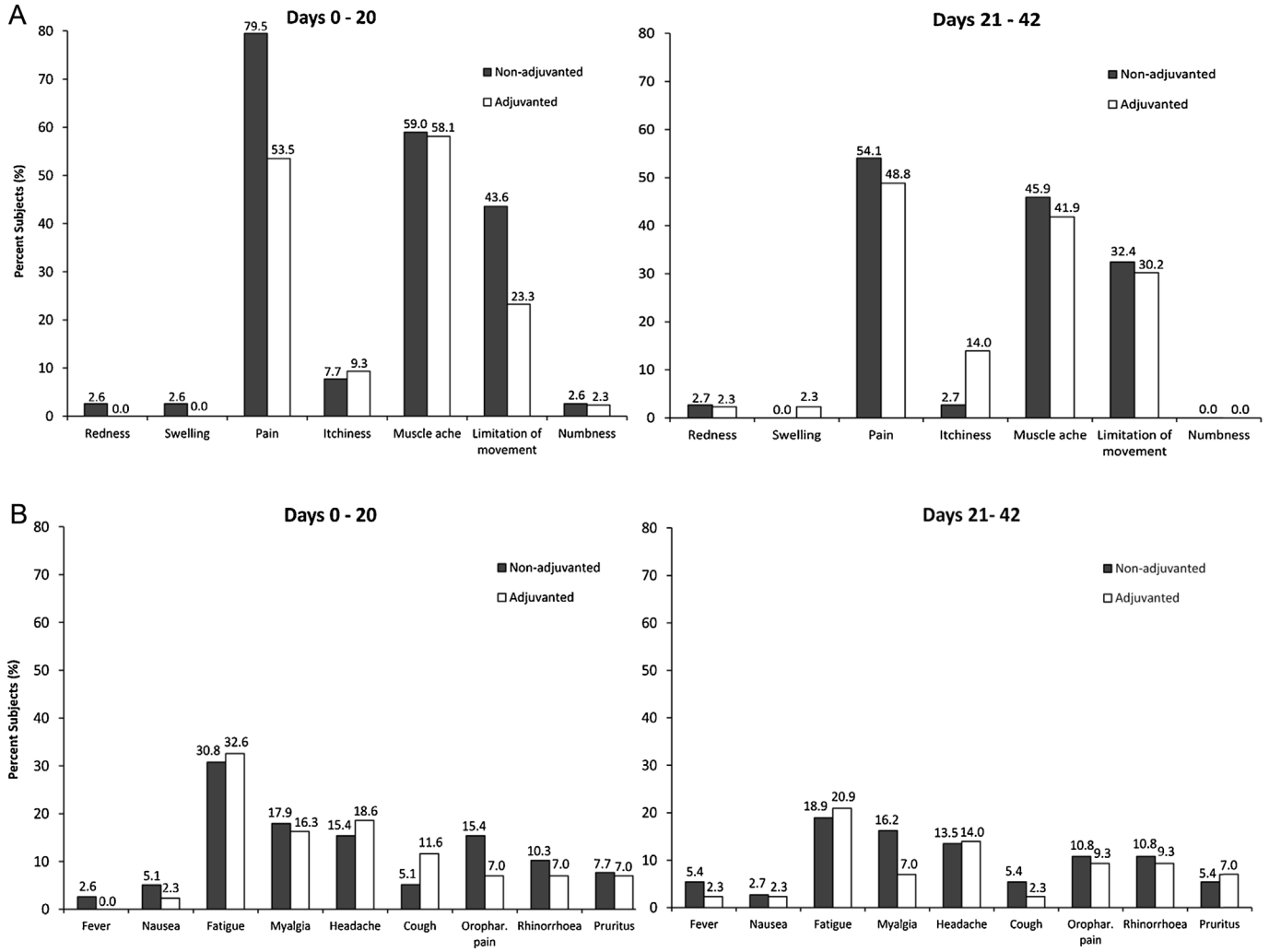

Fig. 2. Adverse events: (A) Incidence of solicited local AEs that occurred after the first and before the second vaccination (left panel) and during the 21 days following the second vaccination (right panel). (B) Systemic (non-solicited) AEs occurring in ≥5% of subjects after the first and the second vaccination are shown in the left and right hand panels, respectively.

Table 3
Summary of antibody mediated immunogenicity.

	Non-adjuvanted (n = 39)			Adjuvanted (n = 43)		
	Day 0	Day 21	Day 42	Day 0	Day 21	Day 42
A/California/07/2009 (H1N1)						
GMT (95% CI)	5.8 (4.9–7.0)	61.0 (35.9–103.5)	70.2 (43.2–114.1)	5.5 (5.0–6.0)	15.0 (10.4–21.4)	33.2 (23.1–47.8)
Seroconversion (95% CI) (%)	NA	62.2 (46.1–75.9)	70.3 (54.2–82.5)	NA	25.6 (14.9–40.2)	51.2 (36.8–65.4)
Seroprotection (95% CI) (%)	NA	66.7 (50.3–79.8)	75.0 (58.9–86.2)	NA	30.2 (18.6–45.1)	53.5 (38.9–67.5)
GMR (95% CI)	NA	10.3 (6.0–17.7)	11.9 (7.1–19.8)	NA	2.7 (2.0–3.8)	6.1 (4.2–8.8)
A/Brisbane/10/2010 (H1N1)						
GMT (95% CI)	6.6 (5.2–8.5)	76.3 (40.4–144.2)	90.4 (52.9–154.3)	6.7 (5.6–8.1)	25.3 (16.3–39.1)	54.3 (36.2–81.6)
Seroconversion (95% CI) (%)	NA	64.9 (48.8–78.2)	73.0 (57.0–84.6)	NA	41.9 (28.4–56.7)	62.8 (47.9–75.6)
Seroprotection (95% CI) (%)	NA	65.7 (49.2–79.2)	74.3 (57.9–85.8)	NA	45.2 (31.2–60.1)	71.4 (56.4–82.8)
GMR (95% CI)	NA	11.3 (6.1–20.9)	13.4 (7.9–22.8)	NA	3.8 (2.6–5.4)	8.1 (5.5–11.8)
A/Georgia/01/2013 (H1N1)						
GMT (95% CI)	6.2 (5.1–7.7)	51.0 (27.0–96.3)	66.3 (37.3–118.0)	6.1 (5.3–7.0)	17.7 (11.5–27.3)	41.6 (27.0–64.2)
Seroconversion (95% CI) (%)	NA	56.8 (40.9–71.3)	64.9 (48.8–78.2)	NA	34.9 (22.4–49.8)	58.1 (43.3–71.6)
Seroprotection (95% CI) (%)	NA	58.3 (42.2–72.9)	66.7 (50.3–79.8)	NA	38.1 (25.0–53.2)	61.9 (46.8–75.0)
GMR (95% CI)	NA	8.2 (4.4–15.0)	10.6 (6.0–18.6)	NA	2.9 (2.0–4.2)	6.9 (4.6–10.1)

seroconversion. Hence, only the non-adjuvanted group met the FDA criterion of a ≥40% lower bound CI for seroconversion. An increase in the percentage of subjects with seroconversion between Day 21 and Day 42 was observed in both groups after boosting. The percentage of subjects with seroconversion was lower in the adjuvanted group than in the non-adjuvanted group on both days. Of the 79 subjects who had baseline HAI titers <40 and HAI titers available on Day 21 and Day 42, 13/43 (30.2%) in the adjuvanted group, and 24/36 (66.7%) in the non-adjuvant group (p = 0.002) showed seroprotection against the strain A/California/7/2009 (H1N1) on Day 21 (Table 3). The GMT was significantly higher (p = 0.013) in the non-adjuvanted group (GMT = 70.2) than in the adjuvanted group (GMT = 33.2).

In addition cross-reactivity of the induced antibodies was evaluated against two drifted influenza strains, A/Brisbane/10/2010 (H1N1) and A/Georgia/01/2013 (H1N1). The immunogenicity against both strains was similar to that demonstrated for A/California/7/2009. The seroconversion rates following two doses of the non-adjuvanted vaccine were 73.0% (95% CI: 57.0 to 84.6%) and 64.9% (95% CI: 48.8 to 78.2%) for the A/Brisbane/10/2010 (H1N1) and A/Georgia/01/2013 strain, respectively, (Table 3) satisfying the FDA criterion of a 40% lower bound CI for seroconversion.

4. Discussion

In this clinical study the bacterially produced pandemic influenza vaccine candidate gH1-Qbeta proved to be well-tolerated and immunogenic in healthy volunteers of Asian ethnicity.

A systematic review of 40 studies with commercially licensed, single dose inactivated influenza vaccines performed between 1990 and 2006 showed a seroconversion rate of 72% for influenza A/H1N1 strains (95% CI: 66% to 78%) with a large variation between individual studies (ranging from 20 to 100%) [33]. Results for non-adjuvanted gH1-Qbeta were comparable, therefore supporting the efficacy of gH1-Qbeta. The antigen dose required (42 μg HA) was higher than the 5 μg shown to be sufficient to achieve seroconversion with the baculovirus-produced VLP vaccine (Novavax Inc.) against the same influenza strain [16]. However, in contrast to the Novavax vaccine and egg-based influenza vaccines the antigen of gH1-Qbeta is based on the globular HA domain only, without lipid bi-layer. The dose (100 μg) was chosen based on ferret efficacy studies [25] and isn't necessarily the lowest efficacious dose. An additional clinical study will be required to establish the lowest dose inducing seroconversion.

In a large randomized controlled trial, comparing an intradermal with an intramuscular influenza vaccine in adults [34], local and systemic reactions were demonstrated with the intramuscular vaccine in 66.3% and 47.9% of subjects, respectively. In our study with the intramuscular gh1-Qbeta we observed a higher incidence of local reactions, especially injection site pain, but a lower incidence of most systemic reactions as compared to the intramuscular influenza vaccine described by Arnou et al. [34]. Overall, adverse events observed were similar in type and range to those described in other influenza vaccine studies [7,16,35].

In this study gH1-Qbeta alone induced higher HAI titer against A/California/7/2009 (H1N1) than in the presence of alhydrogel adjuvant. This is in line with findings with other influenza vaccines where aluminum based adjuvants did not improve or even reduced the immunogenicity of influenza vaccines [36–41], however, these findings were not expected after preclinical efficacy models in mice and ferrets where alhydrogel increased HAI titers or had a neutral effect, respectively [25]. Further studies would be required to ensure that no changes in antigen structure occurred after adsorption to alhydrogel although a research group investigating the effect of aluminum adsorption on antigen structure have not found any changes in the six proteins they have investigated [42,43].

Of interest is the cross-reactivity of the induced antibodies observed against two drifted influenza strains: A/Brisbane/10/2010 (H1N1) and A/Georgia/01/2013(H1N1). All H1N1 strains since the early phase of the pandemic in 2009 have acquired three mutations (P100S, S220T and I338V) in the HA domain compared to A/California/7/2009 (H1N1) without leading to reduced antigenicity of these strains. Although A/Brisbane/10/2010 (H1N1) which acquired additional two mutations (E391K and N142D) compared to A/California/7/2009 (H1N1), was still antigenically similar to A/California/7/2009 (H1N1) using ferret antisera, HAI GMTs against this strain were 53% lower in human sera of subjects vaccinated with Fluvax® (CSL Limited, Australia), a marketed flu vaccine against A/California/7/2009 (H1N1), than against the cognate virus A/California/7/2009 (H1N1) [44,45]. In contrast, after vaccination with gH1-Qbeta, HAI titers against A/Brisbane/10/2010 (H1N1) were comparable to those achieved against A/California/7/2009 (H1N1), indicating a more persistent cross-reactive immunogenicity compared to the egg-based Fluvax®.

Likewise, A/Georgia/01/2013 (H1N1), a representative of a genetically drifted H1N1 strain from early 2013 (FluSurver tool [http://flusurver.bii.a-star.edu.sg]) which has already acquired a total of 11 mutations in the HA domain (P100S, D114N, K180Q, S202T, S220T, A273T, K300E, I338V, E391K, S468N, E516K) compared to the original A/California/07/2009 (H1N1) was recognized similarly as the cognate A/California/07/2009 (H1N1) by the induced antibodies as determined by HAI assay. The fact that this vaccine against A/California/07/2009 (H1N1) shows similar reactivity to two different drifted strains with 5 and 11

mutations, respectively, underscores the quality of the immune response induced and suggests that this vaccine may be protective over several flu seasons confirming the excellent cross-protection found with this vaccine in a mouse model for influenza infection [24].

In summary, the study presented here shows, for the first time, that a fully bacterially produced VLP influenza vaccine is able to induce a strong anti-viral antibody response of high quality and therefore vaccines based on the Qbeta platform are a potential approach for responding to an influenza pandemic. However, to develop this technology for wider use it would be important to establish to what extent this vaccine technology can be used in individuals repeatedly immunized with Qbeta vaccines and whether a B-cell response against the Qbeta component would interfere with subsequent immunizations with different antigens. Once this has been established this novel technology may serve as a new tool in our armamentarium to fight future pandemics and seasonal influenza epidemics.

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Conflict of interest statement

Philippe Saudan is currently employed by Cytos Biotechnology AG and holds stocks and stock options in Cytos AG. Martin Bachmann is a former employee of Cytos AG but is no longer affiliated with Cytos AG. All other authors don't have conflicts of interest.

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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.vaccine.2014.07.011.

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