

RESEARCH PAPER



Immunogenicity and safety of the new MMR vaccine containing measles AIK-C, rubella Takahashi, and mumps RIT4385 strains in Japanese children: a randomized phase I/II clinical trial

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ABSTRACT

Domestic measles, mumps, and rubella combined (MMR) vaccines were discontinued in 1993 in Japan because of the unexpected high incidence of aseptic meningitis. The introduction of an effective MMR vaccine with lower reactogenicity has been expected. A new MMR vaccine (JVC-001) was developed, using mumps RIT4385 strain in combination with Japanese measles AIK-C strain and rubella Takahashi strain (MR) vaccine. An open-label, randomized, phase I/II clinical study was conducted in 100 healthy Japanese children equally randomized to a JVC-001 group and an MR with monovalent mumps vaccine (Hoshino strain) group. Immunogenicity was assessed using a neutralization test (NT) for measles, hemagglutination inhibition (HI) test for rubella, and NT and enzyme-linked immune-sorbent assay (ELISA) for mumps strain with different genotypes (genotype A, B, D and G) on Day 0 and Day 42–56. Solicited and unsolicited adverse events (AEs) were recorded. Seroconversion rates of measles and rubella were both 100%. JVC-001 induced higher immunogenicity against mumps virus genotype G with seroconversion rate of 77.1% (95% confidence interval [CI]: 62.7–88.0%) compared to 65.3% (95% CI: 50.4–78.3%) in the control group. Geometric mean titer (GMT) was 12.5 (95% CI: 8.6–18.3) in the JVC-001 group and 7.1 (95% CI: 5.0–10.1) in the control group. JVC-001 also induced good immunogenicity against other genotypes (A, B and D). There was no apparent difference in the incidence of AEs between JVC-001 and the control groups. JVC-001 is safe and induces effective immunogenicity against measles, mumps, and rubella compared with the currently marketed vaccines in Japan.

ARTICLE HISTORY

Received 13 September 2018
Revised 10 January 2019
Accepted 24 January 2019

KEYWORDS

Immunogenicity; MMR; mumps virus genotype G; neutralizing antibody; RIT4385

Introduction

In Japan, development of domestic mumps vaccine started in 1970's and the Hoshino mumps vaccine strain was first licensed in 1980.¹ By 1990, four different mumps vaccine strains (Urabe, Torii, Miyahara, and NK-M49) derived from different clinical isolates of genotype B were developed.^{2,3} Four live measles, mumps, and rubella combined (MMR) vaccines were developed with different combinations of the mumps vaccine component using Hoshino, Torii and Urabe strains and used in the national immunization programs during 1989–1993.⁴ The Hoshino strain was used, combined with measles AIK-C strain and rubella Takahashi strain.⁵ However, they were discontinued because of an unexpectedly high incidence of aseptic meningitis following vaccination with MMR.^{3–5} Thereafter, monovalent mumps vaccines were used as a voluntary vaccine in Japan. Regarding the risk of aseptic meningitis after monovalent mumps vaccination, Nagai et al.⁶ reported that the incidence of aseptic meningitis after vaccination was 1/27 of that observed in natural infections. It clearly showed the benefit of mumps vaccination, and the introduction of mumps vaccination into Japan national immunization program is highly expected under the current low vaccine coverage.

Deafness after natural mumps infection is a serious complication; initially, mumps deafness was reported in one per 20,000

cases.² However, the incidence of mumps-related deafness was reported to be higher, at one per 1000 cases.⁷ A reduction of mumps-related deafness is one of the targets of vaccine implementation.⁸

Among developed countries, Japan is the only country where the MMR vaccine is not used in national immunization programs because of the associated high incidence of aseptic meningitis.³ Two monovalent vaccines cover only 30–40% of the population, and mumps outbreaks occur every 3–4 years.^{9,10} Mumps virus strains are divided into 12 genotypes based upon the sequence diversity of the small hydrophobic genome region.¹¹ The molecular epidemiology of mumps virus was reported and it was found that circulating wild-type strains were all genotype B in the 1970s but they were of genotypes J and B in the 1980s to 1990s. Genotype G appeared in the 2000s. Genotypes D, I, and L have been isolated sporadically.^{9–13} Nowadays, genotype G is the major circulating genotype worldwide.¹⁰

The Jeryl Lynn (JL) mumps strain of genotype A has been used in MMR vaccine throughout the world. It shows a markedly lower incidence of aseptic meningitis and high immunogenicity.¹⁴ The incidence of mumps has largely decreased since MMR vaccines containing the JL strain started to be used in national immunization programs, even though small mumps outbreaks sporadically occurred among adolescents in the European Union and United States (US).^{15,16}

A safe MMR vaccine containing a mumps strain which shows high immunogenicity is expected in Japan. A major population of the JL strain was cloned as RIT4385.¹⁷ MMR (PriorixTM) containing RIT4385 was developed by GlaxoSmithKline Biologicals (GSK, Warvre, Belgium) and it was reported to induce relatively favorable immune responses as well as a Merck-MMR containing a JL mumps component.¹⁸ In Japan, measles and rubella combined vaccines (MR) were introduced for routine immunization at one and 5–6 years of age. Measles AIK-C strain and rubella Takahashi strains have been used in national immunization programs in Japan with a low incidence of adverse events (AEs).^{3,19} In the present study, a new MMR (JVC-001) vaccine is under development, which consists of MR vaccine produced by Kitasato Daiichi Sankyo Vaccine Co. Ltd. (KDSV, Saitama, Japan) and RIT4385 mumps strain from GSK, and a phase I/II clinical study was conducted with healthy Japanese children.

Results

Study population

One hundred healthy Japanese children were randomized at equal ratio (50 subjects per group) to the JVC-001 or the control group (MR + Mumps), and all of them were vaccinated and completed the study. There was a protocol violation concerning a subject in the JVC-001 group (violation in defined visit allowance) and it was eliminated from immunogenicity analysis. All subjects were evaluated for safety. Background characteristics are shown in Table 1. Mean age was 12.9 ± 1.1 months in the JVC-001 group and 13.3 ± 1.7 months in the control group. There was no difference in sex ratio, height, or body weight.

Immunogenicity against measles and rubella viruses

The results of immunogenicity tests against measles and rubella viruses are shown in Table 2. The seroconversion rate of measles neutralization test (NT) antibody was 100.0% (95% confidence interval [CI]: 94.1–100.0%) and geometric mean titer (GMT) was 43.1 (95% CI: 37.4–49.6) in the JVC-001 group, whereas

a seroconversion rate of 100.0% (95% CI: 94.2–100.0%) and GMT of 39.9 (95% CI: 33.1–48.2) were obtained in the control group.

In the JVC-001 group, the seroconversion rate of rubella hemagglutination inhibition (HI) antibody was 100.0% (95% CI: 94.1–100.0%) and GMT was 91.2 (95% CI: 77.0–107.9), whereas a seroconversion rate of 100.0% (95% CI: 94.2–100.0%) and GMT of 76.6 (95% CI: 64.2–91.5) were obtained in the control group.

Thus, antibody responses against measles and rubella were similar between the JVC-001 group and the control group.

Immunogenicity against mumps virus

The results of immunogenicity against different mumps virus genotypes are shown in Table 3. The seroconversion rate of cytopathic effect (CPE)-NT antibody against mumps genotype A virus was 85.4% (95% CI: 72.2–93.9%) in the JVC-001 group and 56.0% (95% CI: 41.3–70.0%) in the control group. GMT was 19.9 (95% CI: 13.2–29.9) in the JVC-001 group and 5.8 (95% CI: 4.0–8.4) in the control group. The seroconversion rate of CPE-NT antibody titers against mumps genotype B virus, was 85.4% (95% CI: 72.2–93.9%) in the JVC-001 group and 66.0% (95% CI: 51.2–78.8%) in the control group. GMT was 15.8 (95% CI: 10.9–22.8) in the JVC-001 group and 6.8 (95% CI: 4.8–9.6) in the control group.

As for mumps genotype D virus, the seroconversion rate of plaque reduction neutralization test (PRNT) antibody titers was 90.7% (95% CI: 77.9–97.4%), the seroresponse rate was 88.4% (95% CI: 74.9–96.1%) and GMT was 32.1 (95% CI: 19.6–52.4) in the JVC-001 group. In the control group, the seroconversion was 95.8% (95% CI: 85.8–99.5%), the seroresponse rate was 87.5% (95% CI: 74.8–95.3%) and GMT was 31.4 (95% CI: 19.6–50.5). Regarding mumps genotype D, JVC-001 showed an antibody response similar to that obtained in the control group.

In the case of mumps genotype G virus, the seroconversion rate of CPE-NT antibody titers was 77.1% (95% CI: 62.7–88.0%) in the JVC-001 group and 65.3% (95% CI: 50.4–78.3%) in the control group. GMT was 12.5 (95% CI: 8.6–18.3) in the JVC-001 group and 7.1 (95% CI: 5.0–10.1) in the control group. JVC-001 induced higher antibody response against mumps genotype G than the control group.

The seroconversion rate of enzyme-linked immune-sorbent assay (ELISA) antibodies titers against genotype A was 93.8% (95% CI: 82.8–98.7%) in the JVC-001 group and 93.9% (95% CI: 83.1–98.7%) in the control group. GMT was 1745.0 (95% CI: 1250.2–2435.5) in the JVC-001 group and 1400.4 (95% CI: 964.6–2033.0) in the control group.

Table 1. Background of the subjects.

| | JVC-001 (n = 50) | MR+Mumps (n = 50) |
|---------------------------------|---------------------------|---------------------------|
| Mean Age \pm SD | 12.9 ± 1.1 months | 13.3 ± 1.7 months |
| Sex: n, Male/Female | 26/24 | 29/21 |
| Height: Mean, (min-max) | 73.9 cm (67.8–81.0 cm) | 75.2 cm (70.1–82.3 cm) |
| Body weight: Mean, (min-max) | 9.3 kg (7.0–12.0 kg) | 9.6 kg (7.6–11.9 kg) |

Table 2. The immunogenicity against measles and rubella after vaccination.

| | JVC-001 (n = 49) | MR+Mumps (n = 50) |
|--------------------------|----------------------------|---------------------------|
| NT against measles virus | | |
| Seroconversion rate * | 49/49 (100%, [94.1–100%]) | 50/50 (100%, [94.2–100%]) |
| GMT at Day 42 | 43.1, [95% CI: 37.4–49.6] | 39.9, [95% CI: 33.1–48.2] |
| HI against rubella virus | | |
| Seroconversion rate * | 49/49 (100%, [94.1–100%]) | 50/50 (100%, [94.2–100%]) |
| GMT at Day 42 | 91.2, [95% CI: 77.0–107.9] | 76.6 [95% CI: 64.2–91.5] |

*: (Seroconversion rate, [95% confidence interval])

Note: See immunological assessment section for the definition of seroconversion rate. Population parameters of each seroconversion rate were calculated based on each definition with each cut-off value in pre-immunization. Subjects whose assay data was failed to obtain were also eliminated from the calculation.

Table 3. Immunogenicity against different mumps genotypes and ELISA antibodies.

| Mumps NT against | JVC-001 (n = 49) | MR+Mumps (n = 50) |
|---------------------|---------------------------------|--------------------------------|
| Genotype A | | |
| Seroconversion rate | 41/48 (85.4%, [72.2–93.9%]) | 28/50 (56.0%, [41.3–70.0%]) |
| GMT at Day 42 | 19.9, [95% CI: 13.2–29.9] | 5.8 [95% CI: 4.0–8.4] |
| Genotype B | | |
| Seroconversion rate | 41/48 (85.4%, [72.2–93.9%]) | 33/50 (66.0%, [51.2–78.8%]) |
| GMT at Day 42 | 15.8, [95% CI: 10.9–22.8] | 6.8, [95% CI: 4.8–9.6] |
| Genotype G | | |
| Seroconversion rate | 37/48 (77.1%, [62.7–88.0%]) | 32/49 (65.3%, [50.4–78.3%]) |
| GMT at Day 42 | 12.5, [95% CI: 8.6–18.3] | 7.1, [95% CI: 5.0–10.1] |
| Genotype D | | |
| Seroconversion rate | 39/43 (90.7%, [77.9–97.4%]) | 46/48 (95.8%, [85.8–99.5%]) |
| Seroresponse rate | 38/43 (88.4%, [74.9–96.1%]) | 42/48 (87.5%, [74.8–95.3%]) |
| GMT at Day 42 | 32.1, [95% CI: 19.6–52.4] | 31.4, [95% CI: 19.6–50.5] |
| Mumps ELISA | JVC-001 (n = 49) | MR+Mumps (n = 50) |
| Seroconversion rate | 45/48 (93.8%, [82.8–98.7%]) | 46/49 (93.9%, [83.1–98.7%]) |
| GMT at Day 42 | 1745.0, [95% CI: 1250.2–2435.5] | 1400.4, [95% CI: 964.6–2033.0] |

Note: See immunological assessment section for the definition of seroconversion/seroresponse rate. Population parameters of each seroconversion/seroresponse rate were calculated based on each definition with each cut-off value in pre-immunization. Subjects whose assay data was failed to obtain were also eliminated from the calculation.

Vaccine safety

The incidence of solicited local AEs, such as redness, swelling, and pain at the injection site is shown in Table 4. Redness was observed in 19/50 (38.0%, 95% CI: 24.7–52.8%) in the JVC-001 group, 23/50 (46.0%, 95% CI: 31.8–60.7%) at the injection site of control mumps vaccine, and 20/50 (40.0%, 95% CI: 26.4–54.8%) at the injection site of MR vaccine. Local swelling was observed in 5 at the injection site of JVC-001, and 8 and 9 at that of mumps and MR vaccines, respectively. Local pain was observed in 5 at the injection site of JVC-001, and 3 at that of mumps and MR vaccines. These AEs were evaluated as adverse reactions (ADRs). The incidence of unsolicited local AEs/ADRs was similar in the JVC-001 group and the control group.

The incidence of solicited systemic AEs such as fever ($\geq 37.5^{\circ}\text{C}$), parotid/salivary gland swelling, signs indicative of aseptic meningitis, measles/rubella-like rash and other rashes is shown in Table 5. As for fever, AE occurred in 35/50 cases (70.0%, 95% CI: 55.4–82.1%) in the JVC-001 group and in 33/50 cases (66.0%, 95% CI: 51.2–78.8%) in the control group, and ADR occurred in 15/50 cases (30.0%, 95% CI: 17.9–44.6%) in the JVC-001 group and in 8/50 cases (16.0%, 95% CI: 7.2–29.1%) in the control group. A febrile reaction $\geq 38^{\circ}\text{C}$ was observed in 3/50 of the JVC-001 group and 2/50 of the control group (data not shown). As for measles/rubella-like rash, the incidences of AE/ADR were 2/50 (4.0%, 95% CI: 0.5–13.7%) in the JVC-001 group and 3/50 (6.0%, 95% CI: 1.3–16.5%) in the control group. As for other rashes, the incidences of AE were 10/50 (20.0%, 95% CI: 10.0–33.7%) in the JVC-001 group and 11/50 (22.0%, 95% CI: 11.5–36.0%) in the control group and the incidences of ADR were 1/50 (2.0%, 95% CI: 0.1–10.6) in the JVC-001 group and 2/50 (4.0%, 95% CI: 0.5–13.7) in the control group. No parotid/salivary gland swelling, or sign

indicative of aseptic meningitis developed in either group. The incidences of unsolicited systemic AEs/ADRs were similar in the JVC-001 group and the control group.

There was no immediate reaction after vaccination, death, serious AE or AE which caused discontinuation of the study.

Discussion

The RIT4385 strain was established by cloning a major population of JL strain.¹⁷ The efficacy and safety profile of the JL strain have been established. In the US, the incidence of mumps has largely decreased since MMR vaccines containing the JL strain started to be used in the national immunization programs; however, small mumps outbreaks have sporadically occurred.¹⁶ Circulating strains were of genotype G, which is phylogenetically distant from vaccine strains of genotypes A and B, and it brought about discussions on the significance for introducing genotype A mumps vaccine into Japan. The JL strain is genotype A and the serum antibodies raised by the JL strain showed low cross-reactivity against the circulating genotype G strain. The effectiveness of JL strain in preventing mumps infection was 64 to 66% for one dose and 83 to 88% for two doses according to the Cochrane Database.²⁰ Rubin et al.²¹ reported that GMT against genotype G was approximately half of that against the JL strain. Low levels of antibodies did not protect against infection, and Gouma et al.²² assayed the pre-outbreak serum samples after MMR vaccination for NT against genotypes G and D. NT against wild-type mumps virus genotypes G and D was significantly reduced in pre-outbreak samples from infected persons compared with non-infected persons. Zengel et al.²³ reported that there was a significant decrease in the ability of the JL vaccine to produce neutralizing antibody to non-matched viruses.

Table 4. Incidence of solicited local adverse events.

| | JVC-001 | | Mumps | | MR | |
|--------------------|-----------|--------------|-----------|--------------|-----------|--------------|
| | (N = 50) | (95% CI) | (N = 50) | (95% CI) | (N = 50) | (95% CI) |
| Redness (erythema) | 19 (38.0) | (24.7–52.8%) | 23 (46.0) | (31.8–60.7%) | 20 (40.0) | (26.4–54.8%) |
| Swelling | 5 (10.0) | (3.3–21.8%) | 8 (16.0) | (7.2–29.1%) | 9 (18.0) | (8.6–31.4%) |
| Pain | 5 (10.0) | (3.3–21.8%) | 3 (6.0) | (1.3–16.5%) | 3 (6.0) | (1.3–16.5%) |
| Total | 21 (42.0) | (28.2–56.8%) | 23 (46.0) | (31.8–60.7%) | 21 (42.0) | (28.2–56.8%) |

Table 5. Incidence of solicited systemic adverse events and reactions.

| | | JVC-001 (N = 50) | | MR+Mumps (N = 50) | |
|--|------------------|------------------|----------------|-------------------|----------------|
| | | n (%) | 95% CI | n (%) | 95% CI |
| Fever | adverse events | 35 (70.0) | (55.4 - 82.1%) | 33 (66.0) | (51.2 - 78.8%) |
| | adverse reaction | 15 (30.0) | (17.9 - 44.6%) | 8 (16.0) | (7.2 - 29.1%) |
| measles/rubella-like rash | adverse events | 2 (4.0) | (0.5 - 13.7%) | 3 (6.0) | (1.3 - 16.5%) |
| | adverse reaction | 2 (4.0) | (0.5 - 13.7%) | 3 (6.0) | (1.3 - 16.5%) |
| Other rash | adverse events | 10 (20.0) | (10.0 - 33.7%) | 11 (22.0) | (11.5 - 36.0%) |
| | adverse reaction | 1 (2.0) | (0.1 - 10.6%) | 2 (4.0) | (0.5 - 13.7%) |
| Parotid/salivary gland swelling | adverse events | 0 (0.0) | (0.0 - 5.8%) | 0 (0.0) | (0.0 - 5.8%) |
| | adverse reaction | 0 (0.0) | (0.0 - 5.8%) | 0 (0.0) | (0.0 - 5.8%) |
| Signs indicative of aseptic meningitis | adverse events | 0 (0.0) | (0.0 - 5.8%) | 0 (0.0) | (0.0 - 5.8%) |
| | adverse reaction | 0 (0.0) | (0.0 - 5.8%) | 0 (0.0) | (0.0 - 5.8%) |
| Total | adverse events | 38 (76.0) | (61.8 - 86.9%) | 36 (72.0) | (57.5 - 83.8%) |
| | adverse reaction | 18 (36.0) | (22.9 - 50.8%) | 12 (24.0) | (13.1 - 38.2%) |

In the present study, the immunogenicity of those vaccines (RIT4385/genotype A and Hoshino/genotype B) against several mumps virus genotypes, including genotype G, were directly compared in Japanese healthy children. JVC-001 induced higher immunogenicity against wild-type genotype G (seroconversion rate: 77.1%, GMT: 12.5) compared to the control group (seroconversion rate: 65.3%, GMT: 7.1). Although no standard serological assay method or clinically protective NT antibody level has been confirmed,^{24,25} our results suggest that JVC-001 can be expected to show comparable or higher protective efficacy against mumps than monovalent Hoshino vaccine. JVC-001 also showed good immunogenicity against other genotypes (A, B and D).

Due to the limited number of participants, 50 infants in each group, there was no apparent difference in the incidence of local and systemic AEs/ADRs except fever. The JVC-001 group tended to show a higher incidence of fever ($\geq 37.5^\circ\text{C}$) as ADR, but a febrile reaction of $\geq 38^\circ\text{C}$ was observed in 3/50 children in the JVC-001 group and in 2/50 in the control group, suggesting a similar incidence (data not shown).

As for the ADRs related to the mumps component, no parotid gland swelling occurred in this study. It is reported that parotid gland swelling occurred in 0–1.8%, and that the incidence of symptoms related to aseptic meningitis was 0–0.1% following immunization with GSK-MMR containing RIT4385 in several clinical trials.^{18,26,27} The actual incidence must be assessed in a post-marketing study; accordingly, no case of vaccine-associated aseptic meningitis was reported in a 2-year post-marketing study in Germany that involved 1,575,936 children who had received the GSK-MMR vaccine containing RIT4385.²⁷ There was no immediate reaction or serious ADR after vaccination with JVC-001, indicating that JVC-001 is safe and well tolerated.

In conclusion, JVC-001 containing measles AIK-C, rubella Takahashi, and mumps RIT4385 strains was safe and well tolerated and showed comparable or higher immunogenicity against measles, mumps and rubella than the currently available MR and monovalent mumps vaccines used in Japan, suggesting its protective efficacy against these diseases.

Limitations of this study

There are 2 major limitations in this study. First, this was an open-label study where safety assessment was not conducted under blinded condition and thus results might be biased. Second, the number of subjects of this study was limited (50 subjects per group), and no statistical assessment was

conducted. A phase III study is scheduled to assess immunogenicity and safety of this vaccine in a larger population and to carry out a statistical assessment of results under blinded conditions.

Subjects and methods

Study design

This phase I/II clinical study of JVC-001 was an open-label, multicenter (5 sites), randomized, active controlled study to assess the immunogenicity and safety of this vaccine. A single dose of 0.5 mL of JVC-001 was injected subcutaneously in the upper-outer triceps area. As for the control group, 0.5 mL of mumps and MR vaccines was injected subcutaneously at different sites simultaneously. Paired serum samples were obtained immediately before (Day 0) and 6–8 weeks (Day 42–56) after immunization.

This study was conducted according to Good Clinical Practice and applicable local regulations. The study was approved by the Institutional Review Board of each study site and performed according to the Declaration of Helsinki. The clinical trial was registered as JAPIC Clinical Trials Information (JapicCTI-153,031). Written informed consent was obtained from the parent/guardian of the subject before enrolment.

Subjects

One hundred healthy Japanese children (12 to 24 months of age) were randomized at equal ratio (50 subjects per group) to the JVC-001 or the control group (MR + Mumps). The randomization schedule was prepared by using the permuted block randomization method.

Healthy Japanese children who were 12 to 24 months of age at the vaccination were included in this study. Children who had severe acute illness at the vaccination of this study, any history of anaphylactic shock or allergy against the vaccines components of this study and any history of immunization of the vaccines including measles, mumps or rubella virus were excluded from the study.

Vaccine

The JVC-001 vaccine containing measles AIK-C, rubella Takahashi, and mumps RIT4385 strains was formulated and manufactured by GSK. Virus titer follows specification for each strain globally or domestically in Japan, as shown in Table 6; $\geq 10^{3.7}$ 50% cell culture infective dose (CCID₅₀)/0.5 mL AIK-C

Table 6. Comparison of the components of measles, rubella, and mumps vaccine strain.

| | JVC-001 | MR | Monovalent mumps |
|---------|--|---------------------------|--|
| Measles | AIK-C $\geq 10^{3.7}$ CCID ₅₀ | AIK-C ≥ 5000 FFU | – |
| Rubella | Takahashi $\geq 10^{3.0}$ CCID ₅₀ | Takahashi ≥ 1000 FFU | – |
| Mumps | RIT4385 $\geq 10^{3.7}$ CCID ₅₀ | – | Hoshino ≥ 5000 CCID ₅₀ |

strain, $\geq 10^{3.0}$ CCID₅₀/0.5 mL rubella Takahashi strain and $\geq 10^{3.7}$ CCID₅₀/0.5 mL RIT4385 strain. For the control group, marketed MR (Lot number: HF063A) and mumps Hoshino strain (Lot number: LF036A) were used. The titer followed their approved specification: ≥ 5000 focus forming unit (FFU)/0.5 mL AIK-C strain, ≥ 1000 FFU/0.5 mL rubella Takahashi strain and ≥ 5000 CCID₅₀/0.5 mL mumps Hoshino strain.

Immunogenicity assessment

As for NT against measles virus, serum samples were treated at 56°C for 30 min for deactivation. Two-fold serial dilutions of serum samples, starting at 1:4, were mixed with 100 CCID₅₀ of the Toyoshima strain. The mixture was loaded on a monolayer of Vero cells in duplicate. NT antibody titers were determined by 100% inhibition of the appearance of CPEs.²⁸

As for the HI test against rubella virus, serum samples were treated with Kaolin and goose red blood cells (RBCs) to reduce non-specific factors. Two-fold serial dilutions starting at 1:8 were mixed with 4 units of rubella hemagglutinating antigen and the HI titer was determined after the addition of goose RBC.²⁹

NT antibody assay against mumps virus genotypes A, B, and G was performed by 100% inhibition of the appearance of CPE of mumps virus in LSI Medience Corporation (Tokyo, Japan). RIT4385 strain (genotype A), Hoshino strain (genotype B), and Mp/Tokyo 21/2000 (genotype G) were used. Two-fold serial serum dilutions starting from 1:4 were made and mixed with each mumps virus. Serum/virus mixture was applied on Vero cells. CPE was checked manually under microscope.³⁰ NT antibody titers against Mu90/LO1 strain (genotype D) were determined by in-house GSK's PRNT. Two-fold serial serum dilutions starting from 1:2 were made and mixed with Mu90/LO1 strain. The serum/virus mixture was applied on Vero cells. Infected cells were immunodetected with anti-mumps monoclonal antibodies and anti-mouse horse radish peroxidase-conjugated secondary antibodies then stained with TrueBlue. Plaque forming units were measured automatically.³¹ ELISA against mumps virus was conducted with Enzygnost™ (SIEMENS, Germany) using a mumps genotype A strain and according with manufacturer's instructions, at LSI Medience Corporation.

Seroconversion rate was determined based on the percentage of subjects whose antibody titer exceeded the cut-off value on Day 42–56 among those whose antibody titer was below cut-off value (measles: 4, rubella: 8, mumps CPE-NT: 4, mumps PRNT: 2.5 50% virus neutralization endpoint dilution (ED₅₀), mumps ELISA: $\Delta A = 0.1$ on Day 0). Seroresponse rate was determined based on the percentage of subjects whose antibody titer exceeded 4.0 ED₅₀ on Day 42–56 among those whose antibody titer was below 2.5 ED₅₀ on Day 0 for mumps PRNT.

Safety assessment

AEs/ADRs were monitored during the study period up to 6–8 weeks (Day 42–56) after vaccination. Causality of all AEs was assessed by the investigator. AEs which were considered causally related to vaccination were handled as ADRs. AEs/ADRs were collected during the study period using diary cards completed by the subjects' parents/guardians. Solicited local AEs/ADRs were defined as erythema, pain, and swelling at the injection site(s) developed during a first three days (Day 0–3). Solicited systemic AEs/ADRs were fever, defined as increased body temperature $\geq 37.5^\circ\text{C}$ measured at the axilla, measles/rubella-like rash, other rash, parotid/salivary gland swelling, and signs indicative of aseptic meningitis developed from Day 0 to Day 42.

Statistical analysis

The statistical analysis was descriptive only and performed with SAS Software Release 9.2 for Windows.

Abbreviations

| | |
|---------|--|
| ADR: | adverse reaction |
| AE: | adverse event |
| CCID50: | 50% cell culture infective dose |
| CI: | confidence interval |
| CPE: | cytopathic effect |
| ED50: | 50% virus neutralization endpoint dilution |
| ELISA: | enzyme-linked immune-sorbent assay |
| FFU: | focus forming unit |
| GMT: | geometric mean titer |
| GSK: | GlaxoSmithKline Biologicals |
| HI: | hemagglutination inhibition |
| JL: | Jeryl Lynn |
| KDSV: | Kitasato Daiichi Sankyo Vaccine Co. Ltd |
| MR: | measles and rubella combined vaccine |
| MMR: | measles, mumps, and rubella combined vaccine |
| NT: | neutralization test |
| PRNT: | plaque reduction neutralization test |
| RBC: | red blood cell |
| US: | United States |

Acknowledgments

The authors appreciate the following doctors who participated in the phase I/II study: Hiroshi Sakiyama (Sakiyama Pediatric Clinic), Hiroji Okawa (Okawa Children & Family Clinic), Akiyoshi Sasamoto (Seijo Sasamoto Pediatric & Allergy Clinic), Takashi Abe (Abe Child Clinic), and Haruo Kuroki (Sotobou Children's Clinic). The authors also appreciate all members involved in development of JVC-001 at Japan Vaccine Co., Ltd., KDSV, and GSK. The author would particularly like to thank Rie Watanabe and Tadashi Enomoto from KDSV for their support in developing the mumps CPE-NT method.

Disclosure of potential conflicts of interest

Tetsuo Nakayama has received research funds from Daiichi Sankyo Co. Ltd., KDSV, and Japan Vaccine Co., Ltd. Masahiro Eda, Motonori Hirano and Wakako Goto are employees of Japan Vaccine Co. Ltd., which funded this study.

Contributors

TN provided medical advice throughout this study and wrote the manuscript. ME designed the trial and was responsible for the statistical analysis. MH monitored the trial. WG was responsible for

immunological assessment and coordinated manuscript drafting. All authors reviewed and approved the final version.

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