Pandemic Influenza A/H1N1v Monovalent Inactivated Whole Virion Alluminium-adjuvanted Vaccine Refluvac® Clinical Trial Abstract Minimum information:

Title of Trial: Randomized blind placebo-controlled Phase 1 clinical trial on a single dose-increasing application of the inactivated whole-virion influenza vaccine with aluminium (Refluvac®) on volunteers aged 18-60 years.

Clinical Trial registration site if applicable (e.g. ClinicalTrials.gov): N/A

Authors/sponsors: The Republican Government Enterprise "Research Institute for Biological Safety Problems" (RGE RIBSP)/Science Committee of the Ministry of Education and Science, the Republic of Kazakhstan

Study Design (including the phase of clinical trial):
The randomized blind placebo-controlled Phase 1 clinical trial on a single dose-increasing application of the inactivated whole-virion influenza vaccine with aluminium (Refluvac®) was carried out on volunteers aged 18-60 years to assess its safety, immunogenicity and tolerance. The assay involved 54 healthy volunteers. They were Refluvac® vaccinated at a dose of 3.75, 7.5 and 15 µg of hemagglutinin (HA) per person. The Refluvac® vaccine dose was increased by the decisions of the Independent Data Monitoring Committee (IDMC) in compliance with the planned trial design (Table 1).

Table 1: Pattern of the dose increase

<table>
<thead>
<tr>
<th>Dose level</th>
<th>HA content/dose per person</th>
<th>Number of Refluvac® vaccinated volunteers</th>
<th>Number of placebo-injected volunteers</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3.75 µg</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>II</td>
<td>7.5 µg</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>III</td>
<td>15 µg</td>
<td>12</td>
<td>6</td>
</tr>
</tbody>
</table>

The trial schedule is presented in Table 2. The vaccine or placebo was administered to the volunteers on the first day of the trial. Active observation of the volunteers lasted 7 days post vaccination (the day of vaccination and 6 more days). Afterwards, starting from the evening of the 7 day till day 21 of the trial and from day 27 till 42nd day (end of the trial) the volunteers recorded emergence of any symptoms as well as their taking any medication in their self-observation diaries. The trial involved only the volunteers that had HAI antibody titers 1:10 to the influenza virus of A/H1N1v subtype and were seronegative for HIV, hepatitis B and C in ELISA. It was conducted at the Research Institute of Influenza (North-West Department, Russian Academy of Medical Sciences (RAMS), St. Petersburg). The trial was one-centered. The trial record, version 1, was dated March 10, 2010, the changes were not made in it. The total duration of each volunteer's participation in the test was no more than 21 days. The trial included the stage of screening, 1st visit of the initial stage (1st vaccination), visits 2-7 (observation of the volunteers during 6 days after the 1st vaccination), and the fina visit 8 on the 21st day of the trial. The interval between the screening visit and the visit of the initial stage did not exceed 14 days, between the initial and final visits – 21 days.

Table 2: Schedule of the clinical trial

<table>
<thead>
<tr>
<th>Procedures/Examination days</th>
<th>Screening</th>
<th>1 (basic parameters)</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>21 day ± 2 days</th>
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<tbody>
<tr>
<td>Getting informed consent</td>
<td>X</td>
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<td></td>
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<td></td>
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<tr>
<td>Checking parameters of vital symptoms</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X X X X X X X</td>
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<td></td>
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<tr>
<td>Physical examination</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X X X X X X X</td>
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<tr>
<td>Neurological examination</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>X</td>
<td></td>
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<td></td>
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<tr>
<td>Laboratory examination:</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td></td>
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<tr>
<td>Clinical and biochemical blood analysis</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>X</td>
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<tr>
<td>Test for HIV, hepatitis B and C urine examination</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>X</td>
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<tr>
<td>Urine test for pregnancy (females of reproductive age)</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>X</td>
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<tr>
<td>Electrocardiogram</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Injection of the preparation under testing</td>
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<tr>
<td>Sampling for assessment of the immune response</td>
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<tr>
<td>Evaluation of antibody titers in blood</td>
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</table>
**Vaccine subtype:** for influenza A/H1N1

**Virus:** NIBRG-121 xp (NIBSC, Great Britain). Vaccine strain is obtained by the method of reverse genetics and contains HA and NA gens from the virus A/California/7/2009 (H1N1)v and PA, PB1, PB2, NP, M and NS gens from highly reproductive influenza virus donor strain A/PR/8/34.

**Manufacturer:** The Republican Government Enterprise "Research Institute for Biological Safety Problems" (RGE RIBSP)/Science Committee of the Ministry of Education and Science, the Republic of Kazakhstan

**Type (whole virus/subvirion/subunit/live/recombinant/DNA/vector):** whole-virion inactivated

**Adjuvant:** aluminium hydroxide

**Delivery system/site:** intramuscularly

**Doses (antigen and adjuvant, number of doses, intervals between administrations):** the Refluvac® vaccine was injected once intramuscularly into a deltoid muscle (0.5 ml) in dose of 3.75 μg HA (Al³ 0.125 mg) or 7.5 μg HA (Al³ 0.25 mg) or 15 μg HA (Al³ 0.5 mg) per person.

**Study population**

**Number of subjects involved:** 54 volunteers

**Age range:** 18-60 years

**Health status:** healthy volunteers

**Special Inclusion/exclusion criteria:**

**Inclusion criteria:**
- healthy male and female volunteers aged 18-60 years;
- persons with seronegative reaction to the influenza virus A/H1N1 (with HAI antibody titers 1:10);
- written informed consent of a volunteer to participate in the trial.

**Exclusion criteria:**
- allergic reaction to chicken ovalbumin or to any previous vaccination;
- acute illness with fever (>37.0°C);
- chronic alcohol and/or drug abuse in anamnesis;
- severe form of atopy in anamnesis;
- seasonal influenza vaccination in 2009/2010;
- volunteers with clinically significant pathological laboratory findings;
- females with the positive urine test for pregnancy;
- application of immunosuppressive preparations including corticosteroids in the period of 4 weeks before administration of the preparation under study;
- clinically significant affections of kidney, liver, gastrointestinal tract, cardiovascular system, blood system, skin, endocrinial, neurological or immunological diseases in anamnesis;
- leucosis or neoplasms in anamnesis;
- seropositive responses to HIV, hepatitis B and C viruses;
- volunteers after administration of antiviral preparations, immunoglobulins or any other preparation under study, after blood transfusion later than 4 weeks before immunization with the tested vaccine;
- volunteers that took anti-inflammatory medicines later than 2 days before administration of the tested preparation;
- participants of any other test trial during the last 3 months;
- volunteers that cause concern about their sticking to the requirements of the trial or persons with marked physical or mental impairment that may impact the course of the trial.
Clinical Endpoints Assessed

**Reactogenicity and safety assessments:** Undesirable phenomena (local and system reactions), findings of physical examinations, vital symptoms, the data of neurological examinations, results of laboratory analyses (clinical and biochemical blood analysis), electrocardiogram.

**Immunogenicity and effectiveness assessments:** Humoral immune response (levels of seroconversion and seroprotection, geometric mean titer, seroconversion factor) – judging on hemagglutination inhibition test (HAIT) with chicken erythrocytes. Cell immune response – judging on stimulation index of LPC in the reaction of blast transformation of leucocytes and production of cytokines in supernatant of AG-stimulated cells.

Results

**Reactogenicity and safety:**

1. In the course of the trial serious adverse effects and adverse effects of high and mean intensity were not observed.
2. The volunteers vaccinated Refluvac® in dose of 3.75µg HA did not have adverse effects of high and mean intensity.
3. The volunteers vaccinated Refluvac® in dose of 7.5µg HA had adverse effects associated with vaccination in the form of local side reactions of low intensity (3 volunteers had pain at the site of injection) and transitory nature (3 days at most). They disappeared without drug therapy.
4. The volunteers vaccinated Refluvac® in dose of 15 µg HA did not have adverse effects of high and mean intensity. One volunteer had system reaction of mean intensity as short time temperature increase up to 37,8°C after 6 hours post vaccination. The reaction disappeared without drug therapy.
5. Deviations of the results of the clinical and laboratory examination of the volunteers on days 7 and 21 from the findings of the initial examination were not observed irrespective of the vaccine dose.

**Immunogenicity:** Assessment of immunogenic activity of the vaccine at a single administration judging on the results of the HAI test had shown that in the group of volunteers vaccinated with Refluvac® in dose of 3.75µg HA the portion of persons with protective titers was as much as 83.3%; the antibody titer increase factor was 10.7; seroprotection rate was 75%, the mean geometric titers (MGT) of antibodies to the influenza A/H1N1 virus was 53.4.

2. In the group of volunteers vaccinated with Refluvac® in dose of 7.5 µg HA the portion of persons with 4-fold seroconversions was as much as 100%; the antibody titer increase factor was 32.0; seroprotection rate was 75%, the mean geometric titers (MGT) of antibodies to the influenza A/H1N1 virus was 160.0.

3. In the group of volunteers vaccinated with Refluvac® in dose of 15 µg HA portion of persons with protective antibody titers increased from 75% up to 83%. However, we did not observe increase of MGT and antibody titer increase factor comparing with the group of volunteers vaccinated in dose of 7.5 µg HA.

**Conclusion:** The study of reactogenicity, safety and immunogenicity of Refluvac® vaccine in doses of 3.75, 7.5 and 15 µg HA at single intramuscular application to volunteers of 18-60 age showed good tolerance, low reactogenicity and obvious immunogenic activity to influenza virus A/H1N1v. All studied factors of the vaccine meet the requirements of CPMP EMEA and criteris of Federal control service in the sphere of human rights protection MU 3.33.1758-03 in the field of inactivated influenza vaccines production. The obtained results allow conducting II phase of clinical trial of the Refluvac® vaccine in doses of 3.75, 7.5 and 15 µg HA on more volunteers at the age of 18-60.

**Current status of the clinical trial (completed, ongoing, in preparation):** Phase I clinical trial towards assessment of the Refluvac® safety, tolerance and immunogenicity are completed on 19th of July 2010.

**Planned time schedule for next phase of development:** Phase II clinical testing of the vaccine Refluvac® in doses of 3.75 and 7.5 µg HA is scheduled in April 2011.