Pandemic Influenza A/H5N1 Vaccine Kazfluvac® (monovalent inactivated whole-virion vaccine formulated with aluminium hydroxide as an adjuvant) Clinical Trial Abstract

**Title of Trial:** Randomized blind placebo-controlled Phase I clinical trial on a single dose-increasing application of the inactivated whole-virion influenza vaccine with aluminium (Kazfluvac®) 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 I clinical trial on a single dose-increasing application of the inactivated whole-virion influenza vaccine with aluminium (Kazfluvac®) was carried out on volunteers aged 18-60 years to assess its safety, immunogenicity and tolerability. The assay involved 36 healthy volunteers. They were Kazfluvac® vaccinated at a dose of either 7.5 or 15 µg of hemagglutinin (HA) per person. The Kazfluvac® 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 Kazfluvac®) vaccinated volunteers</th>
<th>Number of placebo-injected volunteers</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7.5 µg</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>II</td>
<td>15 µg</td>
<td>12</td>
<td>6</td>
</tr>
</tbody>
</table>

The trial schedule is presented in Table 2. The vaccine of 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 AH1N1v subtype and were seronegative for HIV, hepatitis B and C in ELISA. It was conducted at the Mechnikov’s Research Institute of Vaccines and Sera (Russian Academy of Medical Sciences (RAMS), Moscow). The trial record, version 1, was dated March 5, 2010, the changes were not made in it.

The total duration of each volunteer's participation in the test was 42±3 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), visit 8 (the second vaccination), visits 9-14 (observation of the volunteers during 6 days after the 2nd vaccination) and the final visit 15 on the 42nd 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 – 42±3 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>2-7 days</th>
<th>21± 2 days (2nd vaccination)</th>
<th>22-27 days (6 days post 2nd vaccination)</th>
<th>42±3 days (end of the trial)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Getting informed consent</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Checking parameters of vital symptoms</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X&lt;sup&gt;b&lt;/sup&gt;</td>
<td>X</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Physical examination</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X&lt;sup&gt;b&lt;/sup&gt;</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Laboratory examination:</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X&lt;sup&gt;c&lt;/sup&gt;</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</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>X&lt;sup&gt;c&lt;/sup&gt;</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Test for HIV, hepatitis B and C urinary examination</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X&lt;sup&gt;c&lt;/sup&gt;</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td></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>X&lt;sup&gt;c&lt;/sup&gt;</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Injection of the preparation under testing</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X&lt;sup&gt;c&lt;/sup&gt;</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Sampling for assessment of the immune response:</td>
<td></td>
<td></td>
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<tr>
<td>Evaluation of antibody titers in blood sera by HAI test</td>
<td>X</td>
<td>X&lt;sup&gt;c&lt;/sup&gt;</td>
<td>X&lt;sup&gt;e&lt;/sup&gt;</td>
<td>X&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
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<tr>
<td>Evaluation of antibody titers in blood sera by microneutralization test (MNT)</td>
<td>X&lt;sup&gt;c&lt;/sup&gt;</td>
<td>X&lt;sup&gt;e&lt;/sup&gt;</td>
<td>X&lt;sup&gt;d&lt;/sup&gt;</td>
<td>X&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
<td></td>
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<tr>
<td>Side effects and concomitant therapy</td>
<td>X</td>
<td>X&lt;sup&gt;c&lt;/sup&gt;</td>
<td>X&lt;sup&gt;e&lt;/sup&gt;</td>
<td>X&lt;sup&gt;d&lt;/sup&gt;</td>
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</tbody>
</table>

Notes: "-" before vaccination; "-" after administration of the preparation under testing in 20 min and in 2 h post vaccination

**Vaccine subtype:** for influenza A/H5N1

**Virus:** A/AstanaRG/6:2/2009 (H5N1). The strain was constructed jointly by the Research Institute for Biological Safety Problems/SC of RK ME&S and the Influenza Research Institute (North-Western Branch of RAMS (NWB RAMS), St.Petersburg) by the method of reverse genetics from the highly pathogenic avian influenza strain A/Chicken/Astana/6/05 (H5N1) and the highly reproductive influenza virus donor strain A/PR/8/34 (H1N1) with gene ratio 2:6.

**Manufacturer:** The Republican Government Enterprise "Research Institute for Biological Safety Problems" (RGE RlBSP)/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 Kazfluvac® vaccine was injected twice at the interval of 21 days into a deltoid muscle (0.5 ml) in dose of 7.5 µg HA (Al<sup>3+</sup> 0.25 mg) or 15 µg HA (Al<sup>3+</sup> 0.5 mg) per person.

**Study population**

**Number of subjects involved:** 36 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/H5N1(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;
- vaccination against influenza A/H5N1(participants of the H5N1 vaccine clinical trials in 2006-2007);
- seasonal influenza vaccination in 2009/2010;
- volunteers with clinically significant pathological laboratory findings;
- females with the positive urine test for pregnancy;
- 2 weeks 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 horse erythrocytes and microneutralization reaction.

**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 Kazfluvac® in doses of 7.5 and 15 μg HA had adverse effects associated with vaccination in the form of local side reactions of low intensity (in 95% of cases it was pain at the site of injection) and transitory nature (3 days at most). They disappeared without drug therapy.
3. One system reaction of low intensity that did not require drug therapy was recorded among the volunteers vaccinated with Kazfluvac® in dose of 15 μg HA.
4. The dose-dependent differences in occurrence of local postvaccinal reactions were observed; marked differences in occurrence of adverse effects after the 1st and the 2nd vaccinations were not recorded.
5. Deviations of the results of the clinical and laboratory examination of the volunteers on days 7, 21, 28 and 42 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 (with horse erythrocytes and processing of sera with RDE) in 21 day post vaccination had shown that in the group of volunteers vaccinated with Kazfluvac® in dose of 7.5 μg HA the portion of persons with 4-fold sero-conversions was as much as 66.7%; the antibody titer increase factor was 3.3; seroprotection rate was 16.7%, the mean geometric titers (MGT) of antibodies to the influenza A/H5N1 virus was 16.0. After two vaccinations in the group of volunteers vaccinated with Kazfluvac® in dose of 7.5 μg HA increase of all parameters of immunogenic activity of the vaccine was observed: MGT and antibody titer increase factor were respectively 28.0 and 5.7; seroprotection rate was 66.7%, the portion of patients with 4-fold seroconversion was 75.0%. The first vaccination with high dose (15 μg HA) resulted in 90.9% of cases of 4-fold seroconversion; 8.0 antibody titer increase factor, 66.7% seroprotection rate; MGT of antibodies to the influenza A/H5N1 were 39.0. After two vaccinations with Kazfluvac® in dose of 15 μg HA the increase of most indices of the vaccine immunogenicity were also observed: MGT and antibody titer increase factor reached respectively 52.0 and 10.6; seroprotection rate was 88.9%, the portion of persons with 4-fold conversions was 88.9%.

**Conclusion:** Analysis of the indices of immunogenic activity of Kazfluvac® preparation obtained in HAI test with horse erythrocytes has shown that application of the vaccine in dose of 7.5 μg HA requires double administration of the vaccine, at the vaccine application in dose of 15 μg HA single vaccination may be enough. For making final conclusions it is necessary to obtain the results of the microneutralization test that is recommended by WHO as a key diagnostic test for assessing immunogenic activity of the avian influenza H5N1 vaccines.

**Current status of the clinical trial (completed, ongoing, in preparation):** Phase I clinical trial towards assessment of the Kazfluvac® safety and tolerance are completed. Testing the vaccine immunogenicity is going on.

**Planned time schedule for next phase of development:** Phase II clinical testing of the vaccine Kazfluvac® in doses of 7.5 μg HA and 15 μg HA is scheduled in April 2011.